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# *ALTERNARIA BRASSICAE* AND *A. RAPHANI*: SPORULATION IN CULTURE, AND THEIR EFFECTS ON YIELD AND QUALITY OF RAPESEED

BY

Ⓒ KEITH JACOB DEGENHARDT

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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DEPARTMENT OF PLANT SCIENCE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled, 'Alternaria brassicae and A. raphani: Sporulation in Culture, and Their Effects on Yield and Quality of Rapeseed', submitted by Keith Jacob Degenhardt in partial fulfilment of the requirements for the degree of Master of Science.

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Date October 3, 1973

# ABSTRACT

A study was carried out on the effect of the black spot disease, caused by Alternaria brassicae and A. raphani on yield and quality of rapeseed. Mass inoculations of the varieties Span (Brassica campestris) and Zephyr (Brassica napus) with A. brassicae, A. raphani, and a combination of the two were carried out in the field. A method was developed for producing large quantities of spores for inoculation purposes. With this method, from 2 to 4 million spores per 9 cm Petri plate were produced every two weeks.

In Span and Zephyr, respectively, a severe incidence of A. brassicae reduced yield by 63 and 42%; A. raphani reduced yield by 42 and 34%; and a combination of the two spp. reduced yield by 70% and 41%. Approximately 50% of the yield reductions obtained were due to losses in seed size and weight. The disease also increased significantly the number of pods aborting and caused early maturity of diseased plants. Of further interest was the production by A. raphani and A. brassicae of toxic metabolites which could cause foliar symptoms similar to those caused by the fungus, itself.

The effect of black spot on rapeseed quality was not as large as on yield and its components. The disease affected the protein content of rapeseed to a greater extent than the oil content, with Span having the most consistent reductions. In general, unless the reductions in yield in Span were severe, the oil content was not reduced. The fatty acids in the oil were not strongly affected by black spot. In Span, inoculated with A. brassicae, the palmitic and linoleic acids were increased. But, it appears that the two different fungal species could

affect the fatty acid content of the oil differently. The major glucosinolates in the meal were reduced by the disease.

In general, the changes in the quality parameters could be explained by the stress of early maturity caused by a severe disease incidence. But, changes in the relationships of the parameters studied between the control and severe groups, in the differing levels of resistance of Span and Zephyr, in the differing pathogenicity of _A. brassicae_ and _A. raphani_, and in the differing production of toxic metabolites suggest more complex physiological stresses and deviations could be occurring.

# ACKNOWLEDGEMENTS

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# INTRODUCTION

Rapeseed has been grown for various reasons for at least 2000 years, but it was not until the thirteenth and fourteenth centuries that it was used for its pressed oil. The oil at this stage was mainly used for lamp illumination. In the seventeenth and eighteenth centuries Japan was using rapeseed as an edible oil (Downey and Bolton, 1966).

Rapeseed has been grown in the prairie provinces of Canada for oilseed production since 1942. By 1965 there were over a million acres of this crop in Western Canada, and production reached its highest peak in 1971 at 5.3 million acres.

The two species of rapeseed (*Brassica napus*, Argentine and *B. campestris*, Polish) are grown in varying proportions in the prairie provinces. Manitoba grows 40% of Polish- and 60% of Argentine-type rape. Saskatchewan grows 65% of Polish- and 35% of Argentine-type rape; Alberta grows 95% and 5% of Polish- and Argentine-type, respectively.

Both the Polish- and Argentine-type, are susceptible to various diseases. Plant pathologists across the prairies have noted that black spot of rapeseed, caused by *Alternaria raphani* and *A. brassicae*, was one of the major fungal diseases of rapeseed (Berkenkamp, 1972; Petrie and Vanterpool, 1969; and McDonald, 1959). It affects both the Polish and Argentine types of rape. Dr. G. A. Petrie (1973, private communication) stated that since 1970 the incidence of black spot of rapeseed has doubled annually.

The effect that black spot may have on yield and quality of rapeseed has not been investigated. The major components of yield in rapeseed are number of pods per plant, number of seeds per pod, pod length, and seed size (Zuberi and Ahmed, 1973; Ahmed and Zuberi, 1973). Crushed rapeseed yields approximately 40% oil, 50% oil meal and 10% moisture. Thus, the two major components of economic importance in the seed are the oil content of the seed and the protein content of the meal. The relative values of these components varies, but production of rapeseed in Canada would not be economically feasible without both being present in the expected large amounts.

The quality of the oil of rapeseed depends on the ratio of the four major fatty acids. These fatty acids, making up 98% of the oil, are palmitic, oleic, linoleic and linolenic. The lower the ratio of linolenic acid in the oil, the more desirable it is for the edible oil industry.

The quality of the meal of rapeseed also depends on the amount of glucosinolates present in the meal. The glucosinolates are hydrolyzed by the enzyme myrosinase in the presence of moisture to isothiocyanates and oxazolidinethione. Toxicity of rapeseed meal has been attributed to these isothiocyanates and oxazolidinethione. If myrosinase is inactivated quickly and moisture is kept at a minimum, as in the present extraction methods (pre-press solvent- and solvent-processing methods), the major toxicity problems are avoided. But myrosinase may be introduced from other sources, e.g., it may be naturally produced by swine and bacteria in the digestive tracts of other livestock and poultry. It may also be introduced by feed contaminants. The glucosinolates, themselves, before hydrolysis are toxic to poultry and swine at high levels. The isothiocyanates and oxazolidinethione affect adversely thyroid size, reproductivity and growth rate (reduced feed efficiency) in certain classes of livestock. There are five glucosinolate hydrolysis products that can be found in significant quantities in rapeseed. The three major ones are 3-butenyl isothiocyanate (BI), 4-pentenyl isothiocyanate (PI), and (-)-5-vinyl-2-oxazolidinethione (OZT). The minor ones are 4-methylthiobutyl isothiocyanate (MeSB) and β-phenylethyl isothiocyanate (PhE).

The major purpose of this investigation was to determine the effect of the disease black spot caused by A. raphani and A. brassicae, on the yield and certain quality factors of rapeseed. Since large quantities of spores are required for field and greenhouse inoculations, and since Alternaria spp. tend to sporulate poorly under artificial conditions, it was necessary, as a first step, to develop methods for producing consistently a large amount of inoculum on artificial media.

In observations of the symptomology of black spot of rapeseed there appeared to be a strong correlation of increased floret abortion with increased disease incidence. The object of this study was to determine if infection of Alternaria raphani and A. brassicae caused an increased incidence of floret abortion.

A third objective of this investigation was to determine whether A. raphani and A. brassicae produced toxic metabolites which produce symptoms similar to those caused by infection by these pathogens.

# LITERATURE REVIEW

## The Host

The two species of rapeseed grown in Canada are Brassica napus (Argentine-type) and Brassica campestris (Polish-type). These two species are quite closely related. B. napus is an amphidiploid resulting from crosses between B. campestris and B. oleracea (Downey, 1965). In Western Canada, there has been a rapid changeover of licensed varieties, because of drastic changes in seed quality. In addition to this rapid turnover, there has been a large build-up in rapeseed production (Downey, Pawlowski, and McAnsh; 1970). Rapeseed is now the third or fourth most important annual crop in Canada. It is next to wheat and barley and, depending on the year, either next to or ahead of oats (Downey, Pawlowski, and McAnsh, 1970; Statistics Canada, 1972).

## The Pathogens

Prior to 1950, there was considerable confusion concerning the nomenclature of the various Alternaria pathogens, and the matter of nomenclature is still in a state of flux (Neergaard, 1945; Groves and Skolko, 1945; Taber, 1964; Simmons, 1967; and Lucas, 1971).

A. brassicae was first found by Berkeley in 1836 in England. Kuhn of Germany found A. brassicae on rape in 1855 and described the fungus. Following this description, the fungus was found and widely reported from Europe, North and South America and Asia (Neergaard, 1945). Usually it was found on cabbage and other cruciferous crops and weeds (Neergaard, 1945; Changsri and Weber, 1963; and Taber, 1964).

A. raphani was named and described by Groves and Skolko in 1944. It had been reported earlier but was not named. Neergaard (1945) reported that it had been found in Denmark in 1894. He named the fungus A. matthiola because he found it most commonly on Matthiola incana (common stock) and radish. Taber (1964) stated that A. raphani had a narrower host range than A. brassicae. This had also been previously reported by Changsri and Weber (1963).

In Canada, Groves and Skolko, reported A. raphani only on radish in 1944, and in 1950, Atkinson also found it on radish seed. A. brassicae had been found on rapeseed by Groves and Skolko in 1944. In 1948, Vanterpool found A. brassicae on rapeseed in Saskatchewan. Since 1956, black spot of rapeseed has been reported annually as one of the diseases of rapeseed in the prairie provinces (Can. Plant. Dis. Surv. 1956-1972). In 1963, Vanterpool and Taber reported for the first time the occurrence of A. raphani as a pathogen on rape, and in 1964, they included A. raphani with A. brassicae as the cause of black spot of rapeseed.

Usually these species occur singly in infected tissue, but both species have in some cases been isolated from the same plant (Petrie and Vanterpool, 1969). In Alberta, black spot caused by A. brassicae, was the most prevalent disease on rapeseed in 1971 (Berkenkamp, 1972). The occurrence of A. brassicae and A. raphani could vary from year to year because, according to Vanterpool (1963), A. brassicae was favored during wet years and A. raphani during dry years.

The taxonomic description of A. brassicae and A. raphani was given by Groves and Skolko (1944) and Neergaard (1945). Symptomology of the fungi on their hosts was described by Neergaard (1945), McDonald (1959), Changsri and Weber (1963), and Taber (1964).

## Sporulation on Artificial Media

Changsri and Weber (1963) tested 21 different kinds of media for growth and sporulation of A. raphani, A. brassicae, and A. brassicicola. They found that A. brassicae produced spores more readily than A. raphani on the various media. In general, the more concentrated the leaf decoction the greater was the sporulation. Ionnoidis and Main (1973), working with A. alternata, found that by doubling the concentration of the V-8 media, they increased sporulation. Changsri and Weber (1963), Taber (1963), McDonald (1959), and Atkinson (1950) found that some media enhanced sporulation of A. brassicae and A. raphani.

Lukens (1960) found that light stimulated conidiophore production of A. solani but inhibited conidial production at temperatures above 23°C. When either continuous light or darkness was used, fewer spores were produced than in a diurnal light (Lukens, 1962). In diurnal light, a definite zonation of spore production occurred (Lukens, 1962; Changsri and Weber, 1963; and Taber, 1964). Taber (1964) found that the longer the wave length, the greater was the production of conidia in relation to chlamydospores by A. raphani. Billotte (1963) found that ultraviolet light stimulated sporulation of some Alternaria species.

The optimum temperature for maximum sporulation of A. raphani and A. brassicae was found to be between 23 and 25°C. (Neergaard, 1945; McDonald, 1959; Changsri and Weber, 1963; and Taber, 1964).

Wounding of Alternaria colonies was first shown to stimulate sporulation in A. solani by Rands in 1917. Rands also indicated that dehydration resulting from the scarring of the medium was a factor in increasing sporulation. Ludwig et al. (1962) working with A. solani and Billotte (1963), working with A. brassicae, found that scraping a mature colony, followed by washing with water, and then leaving the plates open to cause drying, greatly enhanced spore production.

Ludwig et al. (1962) found that sporulation of old cultures of some Alternaria spp. was revitalized by single spore transfers. McDonald (1959) reported that single spore transfers of A. brassicae on alfalfa decoction agar (ADA) produced the largest amount of spores. He found that there appeared to be an antagonism between colonies of A. brassicae on ADA, although this phenomenon did not occur on V-8 vegetable agar.

Rose Bengal was first shown by Smith and Dawson (1944) to be able to inhibit the growth of actinomycetes and most bacteria, and to reduce the growth of fungal mycelium. Martin in 1950 verified this work. Rose Bengal was commonly added to media used for isolating fungi in pure culture (Alexander, 1965).

## Yield and Quality of Rapeseed

There was a lack of information on the effect of the black spot disease on the yield and quality of rapeseed. McDonald (1959) studied the effect of A. brassicae on Argentine rape and reported that there was no significant reduction in yield and seed size. Loof (1959) stated that Alternaria spp. were the most important pathogens on cruciferous crops in Europe but gave no information on yield reductions.

The effect of other diseases on yield of other crops is well documented. In the various crops this was commonly attributed to a reduction in seed size, and to a lesser extent on other components of yield (Greaney et al, 1941; Flor, 1944; Peterson et al, 1945; Sackston, 1950; Hartwig and Johnson, 1953; Green and Bendelow, 1961; McFadden et al, 1960; Zimmer and Zimmerman, 1972).

There was a lack of information on the effect of any disease of rapeseed on the quality of oil or meal, although references to the effect of diseases on the quality of some other crops were quite common (Greaney et al., 1941; Peterson et al., 1945; Sackston and Carson, 1951; Zimmer and Zimmerman, 1973).

Sackston and Carson (1951) investigated the effect of pasmo and flax rust on the oil content of flaxseed. They found that both flax rust and pasmo significantly reduced the oil content. In sunflowers, Zimmer and Zimmerman (1972) found that rust, systemic downy mildew, and head rot significantly reduced the oil content.

Two other quality factors in rapeseed were the fatty acid content of the oil and the glucosinolate content of the meal. In 1964, Sims did a study on fatty acid conposition of the oil of rapeseed as it changed from pollination to maturity. Fowler and Downey (1970) did a more complete study on the fatty acid content in low erucic acid material. They found that oil and dry matter accumulation followed sigmoidal patterns, with most of the deposition occurring between 14 and 35 days after pollination. There were four major fatty acids in low erucid acid material: palmitic, oleic, linoleic, and linolenic. The ratio of oleic to linoleic and to linolenic acid was established 21 days after pollination (DAP). The major contributor to oil accumulation in rapeseed was the embryo. It initiated oil synthesis between 14 and 21 DAP. After 21 DAP, palmitic acid dropped in relation to the 18 C-unsaturated acids. Oleic acid increased after 21 days by approximately 10%, while linoleic and linolenic acids remained relatively constant. By 42 DAP, the seed was mature.

There was a lack of information on the effect of any disease on the fatty acids of the oil of rapeseed. Sackston and Carson (1951) showed that different diseases have different effects on the fatty acid composition of flaxseed. They found that flax rust increased, while pasmo decreased the iodine number of the oil. Iodine number of the oil was a measure of the degree of unsaturation of the oil (a change in the amount of linolenic acid present in flaxseed changes the iodine number). Zimmer and Zimmerman (1972) found that downy mildew, sunflower rust, head rot, and verticillium wilt had no significant effect on the fatty acids of the oil of sunflower.

Information on the effect of diseases on the glucosinolate content of rapeseed was also lacking. Kondra and Downey (1969) described the changes in glucosinolate content of developing rapeseed. The study was done on the three major glucosinolate hydrolysis products. Argentine varieties, in general, had a higher glucosinolate content than Polish varieties. The relative figures for the Argentine variety, Nugget, were: BI--1.22, PI--0.14, and OZT--6.84 compared to Polish variety, Echo, with BI--1.52, PI--2.00, and OZT--2.04. Between 16 and 28 DAP, levels of all compounds increased rapidly. After 28 days, accumulation continued at a decreased rate. The build-up of these hydrolysis products corresponded closely to the pattern of embryo growth and development. The build-up of the glucosinolates continued until the seed was mature.

## Floret Abortion

Vaartnou and Tewari (1972) reported that *Alternaria alternata* caused floret abortion in rapeseed. However, they did not present any data to support this observation. Thus, information on the effect of *Alternaria* spp. on floret abortion of rapeseed was lacking. Sackston (1950) found that pasmo caused a decrease in branching and a smaller seed set in flax. Dunleavy and Weber (1967) found that soybean yield loss caused by brown stem rot was due to a 64% reduction in seed number and a 36% reduction in seed size. Harper and Pittman (1971, private communication) did a study on the effect of *Albugo cruciferarum* on Span rape. They found that the per cent yield loss was proportional to the per cent of branches with staghead symptoms.

## Toxin Study

Several plant toxins have been isolated from culture filtrates produced by species of *Alternaria*. These were all of relatively low molecular weight, non-enzymatic compounds ranging from small peptides to simple phenols (Templeton, 1972). Recently, in Japan, evidence has been accumulating that a host specific enzymatic toxin may be involved in the action of *A. kikuchiana* on pear (Torikata, 1972, private communication).

The role, if any, of *Alternaria* toxins, during pathogenesis was not yet clear, but there were convincing arguments that some of them participate in symptom development at certain times during infection and incubation. The studies of *A. kikuchiana* on pears have shown that pathogenecity was strongly correlated with toxin production (Torikata, 1967).

*A. alternata* exhibited a great deal of variation in plant infections (Simmons, 1967; Lucas, 1971). A group of toxins have been isolated from *A. alternata*. Fulton *et al.* (1965) showed that *A. alternata* produced a metabolite that caused irreversible chlorosis of the coyledons of developing cotton seedlings. The metabolite was later isolated, purified, and named tentoxin. *A. brassicae* and *A. raphani* did not produce tentoxin. Taber (1964) implied in her thesis that *A. brassicae* and *A. raphani* may produce metabolites that are toxic to rapeseed, but did not investigate this possibility. Husain and Thakur (1966) showed that a culture filtrate of *A. brassicae* induced severe wilting and the appearance of water soaked spots in yellow mustard cuttings within 12 hours. There was a lack of information as to whether *A. brassicae* and *A. raphani* produce metabolites that were toxic to rapeseed.

# MATERIALS AND METHODS

## Sporulation on Artificial Media

### Fungi

*Alternaria brassicae*, used in this study, was isolated from a black spot stem lesion on rapeseed collected in the Lamont, Alberta area. Identification of this isolate was confirmed by Dr. G. A. Petrie, Canada Department of Agriculture Research Station, Saskatoon.

*A. raphani* was obtained from Dr. G. A. Petrie. It was isolated from rapeseed seed produced in the Meadow Lake, Saskatchewan area.

Both species were maintained on V-8 nutrient medium during this investigation. V-8 vegetable juice was a mixture of various vegetables, seasonings, and vitamin C made by Campbell Soup Company Ltd. Miller (1955) was one of the first researchers to recommend it as a general-purpose medium for fungi and bacteria.

### Nutrient Media

The following nutrient agar media were used to determine their effect on the sporulating ability of *A. brassicae* and *A. raphani*.

V-8: 200 ml V-8 vegetable juice, 0.75 g $CaCO_3$, 20 g Difco agar, 1000 ml distilled, demineralized water;

V-8 RB: V-8 with 0.01% streptomycin and 0.004% Rose Bengal added;

MYA (Malt Yeast Agar): 5 g Difco malt extract, 5 g Difco yeast extract, 15 g dextrose, 15 g Difco agar, 1000 ml distilled, demineralized water;

Sach's agar medium: 1.0 g $Ca(NO_3)_2$, 0.25 g $K_2HPO_4$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeCl_3$, 4.0 g $CaCO_3$, 20 g Difco agar, 1000 ml distilled, demineralized water;

PYE (phytone yeast extract agar): 10 g phytone peptone, 5 g yeast extract, 40 g dextrose, 0.03 g streptomycin, 17 g Difco agar, 1000 ml distilled, demineralized water;

MA (malt agar): 25 g malt extract, 17 g Difco agar, 1000 ml distilled, demineralized water.

Twenty-five ml of agar medium was poured into a 9 cm Petri plate and, after solidifying, the medium was seeded with spores scraped from the middle of the colony of *A. brassicae*, or by mycelium scraped from the leading edge of the colony of *A. raphani*. In both methods the plates were with a minimum amount of mycelium. The cultures were incubated at 23°C in 16 hours light with an intensity of 150-200 foot-candles of incandescent light, followed by 8 hours of darkness.

The extent of sporulation was determined 14 days after seeding. The yield of spores was determined by pouring 10 ml of water over each culture, scraping the colony with a wire hook to dislodge spores, and using an Improved Neubauer haemacytometer to obtain the spore counts. Each test was repeated 3 times and 2 readings were taken for each test. The relative yield of spores on the different media were rated on a scale of 0 to 5, with 5 representing the highest yield and 0 representing no spores. The numbers in the scale in terms of the approximate number of spores per ml were as follows: 1 = 100; 2 = 1000; 3 = 10,000; 4 = 100,000; 5 = 200,000.

## Seeding Load of Spore Inoculum

The same methods as described under the heading "Nutrient Agar" were used, with the following changes:

1) only V-8 RB medium was used,
2) the medium was seeded with 1, 2, $2 \times 10^1$, $2 \times 10^2$, and $2 \times 10^3$ spores per ml of *A. raphani* and *A. brassicae*.

## Rose Bengal Concentrations

The same methods as described under the heading "Seeding Load of Spore Inoculum" were used, with the following changes:

1) only single spores of the respective fungi were used to seed the plate,
2) the V-8 had concentrations of 0, 0.002%, 0.004%, 0.008%, and 0.016% Rose Bengal added to the media.

## Temperature

The same methods as described under the heading "Seeding Load of Spore Inoculum," with the following changes were used:

1) only single spores were used to seed the plates,
2) the fungi were cultured at temperatures of 15, 17, 20,

23, 24 25°C.

### Light

The same methods as described under the heading "Temperature," with the following changes, were used:

1) only a temperature of 23°C was used,
2) light regimes were: a) continuous light, b) continuous darkness, c) 16 hr light and 8 hr darkness, and d) 8 hr light and 16 hr darkness. Light at all times refers to an intensity of 150-200 foot candles of incandescent light.

### Depth of Agar Medium

The same methods as described under the heading "Light" were used, with the following changes:

1) the fungi were cultured on 25 ml or 15 ml of V-8 RB, and
2) the cultures were grown in 16 hr darkness and 8 hr light.

### Wounding of the Culture

The same method as described under the heading "Depth of Agar Medium" was used, with the following changes:

1) only plates containing 15 ml of V-8 RB medium were used, and
2) after six days of growth half of the cultures were wounded by scraping with a stiff wire loop, and on the following days after wounding, spore concentrations were determined for wounded and unwounded cultures up to 14 days after seed-

ing.

## Age of Culture

The methods were the same as described under the heading "Depth of Medium," with the following changes:

1) only plates with 15 ml of V-8 RB medium were used,

2) the fungi were grown on V-8 RB for 19 days, and

3) spore counts were made each day with a haemacytometer.

## Effect of A. brassicae and A. raphani on Yield and Quality of Rapeseed

### Varieties of Rapeseed

Two rapeseed varieties were used: Span, a Polish type, *Brassica campestris*, and Zephyr, an Argentine type, *B. napus*. Span is an early variety that matures in about 90 days, and has an average oil and protein content of approximately 39 and 42%, respectively. It is cross-pollinating. Zephyr, a late variety, matures in about 120 days, and has an average oil and protein content of approximately 40 and 41%, respectively. It is self-pollinating.

### Experimental Design

A randomized block design, with each treatment replicated 4 times, was used. There were 8 rows per plot, 5.01 meters in length and spaced 20.5 centimeters apart. The treatments were: 1) non-inoculated plots (control), 2) inoculated with *A. brassicae*, 3) inoculated with *A. raphani*, and 4) inoculated with both species of *Alternaria*.

### Treatments

Each replicate consisted of the 8 row plot of a rapeseed variety bordered with 4 guard rows of the same variety. An inoculation treatment consisted of spraying the central area of 2.44 meters x 0.92 meters in each plot. An inoculum load of 30,000 to 45,000 spores per ml was applied with an air powered paint sprayer, using 1 atmospheric pressure. Two hundred ml of spore suspension were applied per replicate. For the plants treated with the combination of both species a 1:1 suspension made up to 30,000 to 45,000 spores/ml of A. brassicae and A. raphani was used.

All inoculations were carried out between 8:00 and 10:30 p.m. to take advantage of a favorable dew-factor and lower evaporation rate.

## Inoculations

Both varieties of rapeseed were inoculated at 36, 46, 53, and 62 days after seeding. Zephyr was inoculated an additional time at 89 days after seeding.

The stage of development of each variety at the time of inoculation was as follows (according to Berkenkamp's growth stage key, 1973):

1) 36 days after seeding
   Span--first flowers opening on terminal bud, 3.0;
   Zephyr--terminal bud present, 2.0;
2) 46 days after seeding
   Span--lower pods elongating, 3.2;
   Zephyr--buds yellowing, 2.4;
3) 53 days after seeding
   Span--seeds enlarging, all buds open, 3.4;
   Zephyr--peduncle elongating, many flowers open, 3.1;
4) 62 days after seeding
   Span--seeds in lower pods green, opaque, 4.1;

Zephyr--lower pods starting to fill, 3.3;

5) 89 days after seeding

Zephyr--seeds in lower pods green, opaque, 4.1.

## Weather

Louvet and Billotte (1963) found that a succession of wet and dry periods were the most favorable for abundant *Alternaria* spp. spore production. These spores were most commonly disseminated by wind and rain. Infection occurred most readily when free water was present. Under these conditions infection occurred in less than 6 hours.

The weather of the spring and summer of 1972 met these conditions remarkably well. There was above normal precipitation which occurred gradually over the spring and summer months. Thus, the growing season of 1972 was an extremely favorable one for the establishment of a high incidence of *Alternaria* diseases on rapeseed.

## Harvesting Methods

Span and Zephyr were harvested 96 and 125 days after seeding, respectively. Two methods of harvesting were used:

1) Bulk method--Two rows in each locus of infection or in the centre of the controls were harvested and called the bulk-harvest. Plants in this area were moderately to severely diseased. Controls had only a trace of disease and were, therefore, considered healthy;

2) Disease-ranked selection method--plants were grouped into four categories based on the extent of disease development. Three samples, each consisting of five plants, were taken from each plot. The plots were replicated 4 times and, therefore, 12 samples were obtained per rating. A study on disease-ranked material infected with *A. brassicae*, *A. raphani*, and the combination of both species was done. A description of the four disease-ranked categories was as follows:

1) Control--none or few superficial stem lesions, no lesions girdling the stem; pods with few superficial lesions;

2) Slight--at least 3 lesions per branch, large and sunken pod lesions were common; floret abortion greater than that occurring on the control plants by natural pruning;

3) Moderate--stem and pod lesions of the sunken type quite common, with some stem lesions girdling the stem; floret abortion markedly increased over the control;

4) Severe--stem and branch girdled in at least 2 places by sunken lesions, shallower stem and branch lesions occurred every 7 to 15 centimeters; pod lesions were large, sunken, and covering one-quarter to one-half of the pod surface; pod peduncles often lesioned; floret abortion about twice as common as in plants rated slightly diseased.

All determinations for yield and quality were done on two kinds of material, i.e., bulk-harvested and disease-ranked selected plants.

## Methods of Analysis

### Oil Content Analysis

Oil content was analyzed with a wide-line nuclear magnetic resonance (NMR) spectroscope, Mk. 2, from Newport of North America. The technique employed was a modification of one used at the Canada Department of Agriculture, Saskatoon. The bulk samples were analyzed using a 26 g subsample of the harvested material. The disease-ranked were analyzed using a 1.5 g subsample of the harvested material.

### Protein Content Analysis

The protein content of the seed was analyzed by the AACC Method 46-12 method (Watson, 1972, private communication). The percentage of protein of the meal was calculated by using the formula:

$$\text{protein of the meal (\%)} = \frac{\text{protein content of the seed sample (\%)}}{\text{100 - oil content of the sample (\%)}} \times 100$$

### 1000-Kernel Weight Analysis

One thousand seeds obtained from the bulk-harvested and disease-ranked method of harvesting were counted out and weighed.

## 1000-Seed Volume Analysis

The seed obtained for the 1000-kernel weights was used for measuring 1000-seed volumes. The seed was poured into a graduated burette containing a known volume of 70% ethyl alcohol. The quantity of liquid displaced by the seed represented the volume of the 1000 seeds.

## Seed Density Analysis

This value was obtained by dividing the 1000-kernel weight of a sample by its 1000-seed volume.

All of the above analyses were done on all the field harvested material. The glucosinolate and fatty acid analyses were determined on the bulk-harvested material and the disease-ranked material infected with A. brassicae.

## Fatty Acid Analysis

The relative quantity of the 4 major fatty acids of the rapeseed oil was determined by the method described by Downey and Craig (1964). A Hewlett Packard 5750 gas chromatograph was used for this purpose.

## Glucosinolate Analysis

The quantities of the glucosinolate hydrolysis products 3-butenyl isothiocyanate (BI), 4-pentenyl isothiocyanate (PI), 4-methylthiobutyl isothiocyanate (MeSB), β-phenylethyl isothiocyanate (PhE), and (-)-5-vinyl-2-oxazolidinethione (OZT) were determined by the method described by Youngs and Wetter

(1967). (The abbreviated forms of the glucosinolate products will be used henceforth.) A Varian Aerograph Series 1200 gas chromatograph was used for this purpose. Ultraviolet spectrophotometry was used for the OZT determination.

## Methods of Statistical Analyses

Analyses of variance was carried out on the yield and quality data using a randomized block method.

Simple correlations in a matrix for the variables were calculated in order to determine how strongly related the variables were to each other. The correlations were set up for the bulked material of Span and the disease-ranked material of Span inoculated with A. brassicae under the following groupings:

1) Yield, % oil of the seed (oil), % protein of the seed (protein S), % protein of the meal (protein M), 1000-seed weights (1000 kwt), seed volume, and seed density.
2) Yield, oil, and the fatty acids (palmitic, oleic, linoleic, and linolenic acid).
3) Yield, protein S, protein M, and the glucosinolates (BI, PI, MeSB, PhE, OZT--refer above).

## Effect of *A. brassicae* and *A. raphani* on floret abortion and floret numbers

Seeds of the varieties Span and Zephyr were germinated on wet filter paper in 9 cm Petri plates. After 5 days the seedlings were transferred to plastic pots containing a 3:2:1 soil mixture (3 black loam: 2 peat: 1 sand). Three seedlings were planted per 15.2 cm pot. A 16-20-0 fertilizer was applied at the rate of 112 kg per hectare once a month. Eight replicates were used.

The plants were grown in a greenhouse where the temperature ranged from 20°C during the day to 17°C at night. Twenty-four hours prior to infection the plants were transferred to a growth chamber where the temperature was 21°C and the relative humidity was maintained at 85-95 per cent.

The plants were inoculated with *A. brassicae* or *A. raphani* using a DeVilbiss No. 121 atomizer and applying 50 ml of inoculum suspension containing 30,000 to 45,000 spores/ml.

Inoculation of the plants was begun when flowering was initiated, and repeated 4 times at 1-week intervals. Control plants were treated the same as the inoculated ones, with the exception that only distilled water was used to spray them.

## Toxic Metabolites

Three fungal species were used: *A. brassicae* and *A. raphani*, pathogens on rape, and *A. humicola*, a saprophyte commonly occurring on rotten fruit. Each fungus was grown in Petri dishes, 15 cm in diameter containing 100 ml of V-8 RB liquid medium. Each medium was seeded with one ml of spore suspension containing approximately five spores.

The fungi were incubated for 2 weeks at 23°C in a standing culture. The cultures produced a thick mat of mycelium on the surface of the medium. The fungi were harvested by pouring the contents of each Petri plate into a Waring blendor and macerating for one minute. The resulting suspension was filtered through 2 layers of cheesecloth and centrifuged at 15,000 rpm for 45 minutes. The supernatant material was then passed through a series of millipore filters of sizes 3, 1.2, and 0.45 microns. Approximately 250-300 ml of sterile filtrate were obtained from 4 Petri plates.

The controls consisted of sterile water and non-inoculated V-8 RB liquid medium. The non-inoculated liquid medium was processed in the same manner as the inoculated medium. Half of the liquid material was used without further processing, while the remaining half was autoclaved for 20 min. Thus, there were 8 different treatments as follows:

1) *A. brassicae* filtrate (autoclaved)
2) *A. raphani* filtrate (autoclaved)
3) *A. humicola* filtrate (autoclaved)
4) V-8 RB liquid medium filtrate (autoclaved)
5), 6) and 7) as 1, 2, and 3 above but not autoclaved
8) distilled, autoclaved water.

The filtrates were assayed in 2 ways for the presence of toxic metabolites:

1) Germinating seed--seeds of varieties Span and Zephyr were germinated in a large Petri dish, 100 x 80 mm, lined with moist filter paper on the top and bottom. The bottom filter paper was kept moist by the application of the various filtrates or distilled water while the top one was moistened with distilled water. The seedlings were examined after 7 days.

2) Detached leaves--the lower leaves of 35 to 45-day old plants of Zephyr were detached at a point near the stem. These leaves were immediately immersed into test tubes each containing 20 ml of the filtrate or distilled water. The leaves were held in place with a cotton plug surrounding the petiole and inserted into the tube opening.

Each treatment was replicated 3 times and the entire experiment was repeated 3 times. The tubes with the leaves were then placed in a growth chamber for 7 days at a temperature of 20°C and 16 hours light alternating with 8 hours darkness. The leaves were compared to leaves 7 days after inoculation with _A_. _brassicae_ and _A_. _raphani_.

# RESULTS

## Sporulation on Artificial Media

### Nutrient Agar Media

The highest yield of spores of *A. brassicae* and of *A. raphani* was obtained on V-8 agar medium amended by 0.004% Rose Bengal (V-8 RB) (Table 1). Cultures on this medium grew moderately well in diameter and produced spores abundantly on a moderate amount of mycelium. This type of growth also occurred on V-8, and to a lesser extent on ADA media. In all other media, except Sach's mycelial growth was heavy but sporulation was limited. Growth on Sach's medium was very sparse and consequently spore production was also sparse.

There was a considerable amount of variability in spore counts between determinations on the same medium but the averages of these counts could be accurately categorized into the ranges indicated by the 0 to 5 scale.

Table 1. Effect of different nutrient agar media on yield of spores of *Alternaria brassicae* and *A. raphani*

| Medium | Relative yield of spores, 0-5<br>*A. brassicae* | <br>*A. raphani* |
|---|---|---|
| V-8 | 2 | 3 |
| V-8 RB | 3 | 3 |
| MYA | 1 | 1 |
| Sach's | 1 | 1 |
| PSA | 2 | 1 |
| PYE | 0 | 1 |
| MA | 1 | 1 |
| ADA | 3 | 2 |

## Seeding Load of Spore Inoculum

The effect of seeding load on the yield of A. brassicae and A. raphani was shown by the normal hyperbolic curve on a semi-logarithmic scale (Fig. 1). The highest spore yield was obtained when a single spore was used to seed the medium. Even two spores per plate reduced the yield sharply, i.e., by 50% and 65% for A. brassicae and A. raphani, respectively. Further increases in seeding spore load continued to reduce the yield until it stabilized at a spore seeding load of approximately 100 spores/ml for A. raphani, and approximately 200 spores/ml.

## Rose Bengal Concentration

The maximum yield of spores of A. brassicae and A. raphani was obtained in the medium with a 0.004% concentration of Rose Bengal (Fig. 2). In higher concentrations of Rose Bengal, the diameters of the colonies were reduced and the colonies had a darker coloration with less aerial mycelium. In concentrations below 0.004% Rose Bengal, the colony diameter increased, aerial mycelium increased, and colony color was much lighter.

## Temperature

The maximum yields of spores of A. brassicae and A. raphani were obtained at a temperature range of 20-23°C (Table 2). There was a sharp reduction in spore yield at the lower temperatures.

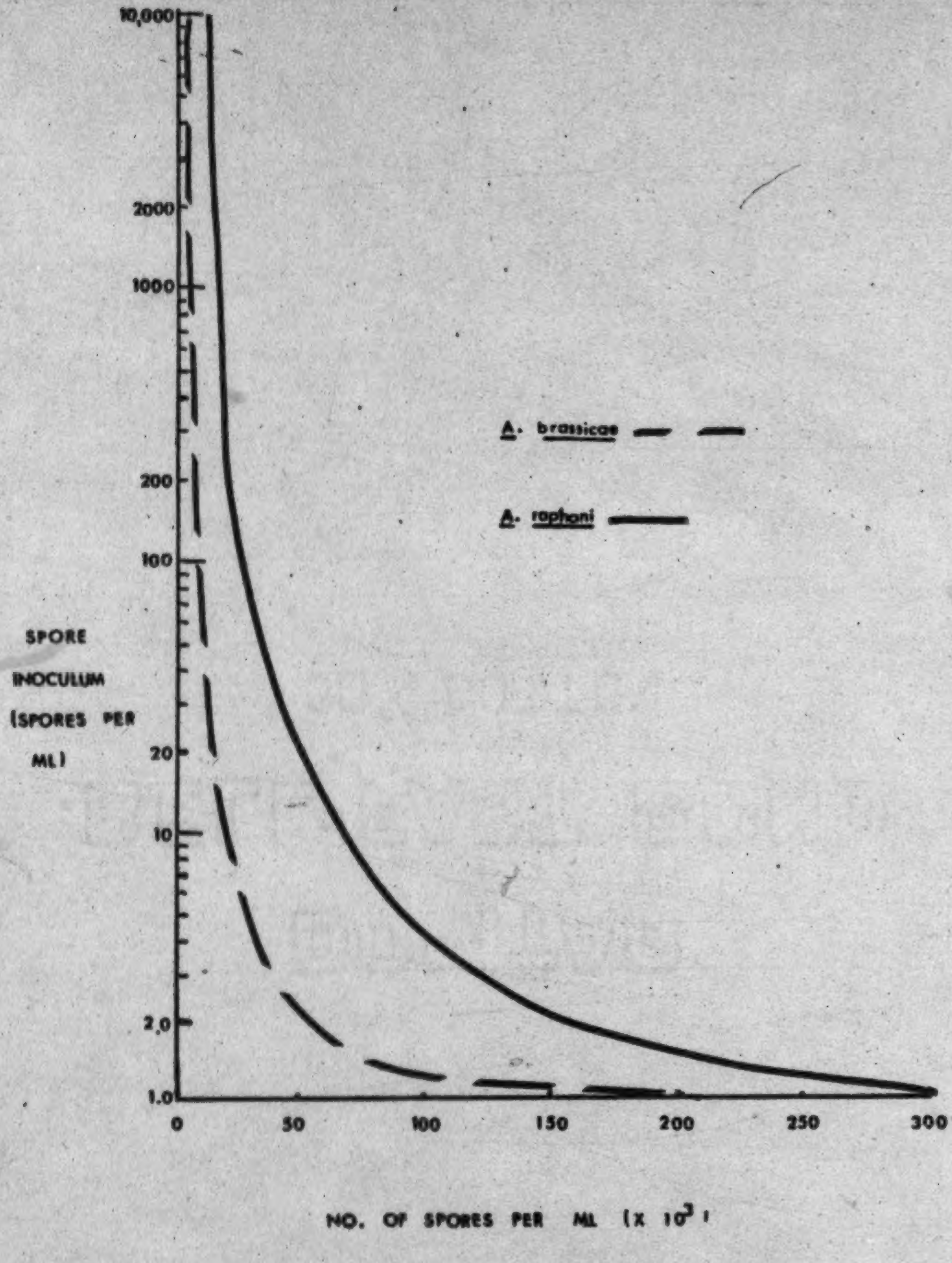


Fig. 1 The effect of inoculum concentration on yield of spores of Alternaria brassicae and A. raphani

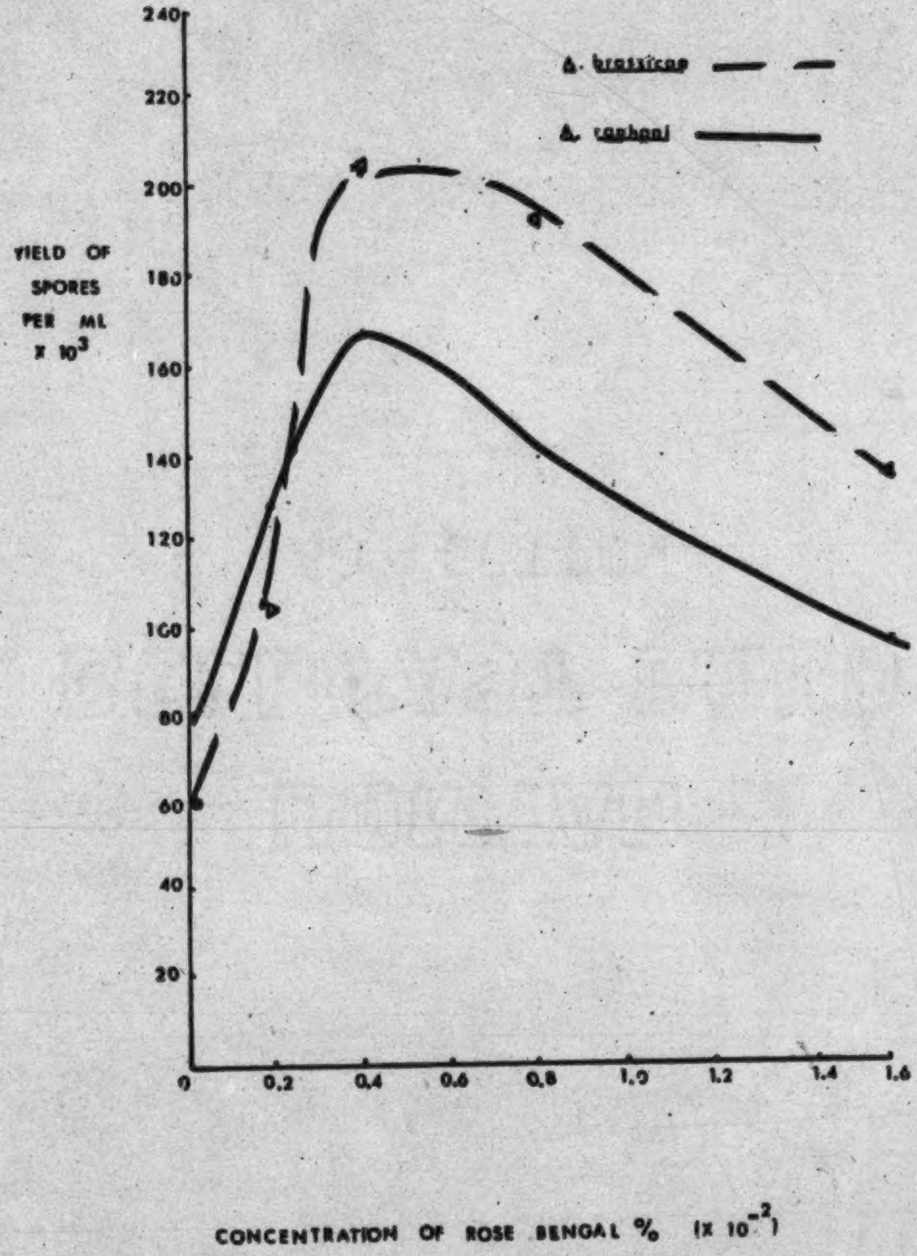


Fig. 2 The effect of concentration of Rose Bengal in V-8 agar medium on yield of spores of *Alternaria brassicae* and *A. raphani*

Table 2. Effect of different temperatures on yield of spores of A. brassicae and A. raphani grown on V-8 RB agar medium

| Temperature ($^{\circ}$C) | Relative yield of spores, 0-5<br>A. brassicae | A. raphani |
|---|---|---|
| 15 | 1 | 1 |
| 17 | 2 | 3 |
| 20 | 5 | 4 |
| 23 | 5 | 4 |
| 25 | 4 | 3 |

## Light

Diurnal light conditions enhanced spore yield to the largest extent, and a regime of 8 hours light and 16 hours darkness favored the highest production of spores (Table 3). Continuous light suppressed spore production more than continuous darkness for *A. brassicae*, while the opposite was true for *A. raphani*. In addition, in continuous darkness, *A. raphani* produced a larger proportion of chlamydospores to conidial spores, while in the other light regimes, the opposite occurred.

Table 3. Effect of different light regimes on yield of spores of A. brassicae and A. raphani grown on V-8 RB agar medium

| Light Regime | Relative yield of spores, 0-5 | |
|---|---|---|
| | A. brassicae | A. raphani |
| Continuous darkness | 3 | 3 |
| Continuous light | 1 | 3 |
| 16 hr light, and 8 hr darkness | 5 | 4 |
| 8 hr light, and 16 hr darkness | 5 | 5 |

## Quantity of Agar Medium

Spore production was increased by approximately 1 1/3 times in A. brassicae and by 2 times in A. raphani in a shallow layer of 15 ml of media as compared to a thicker layer of 25 ml of media (Table 4). The shallow layers of media showed initial signs of drying at 14 days, as indicated by hardening and curling up at the edges of the V-8 RB medium.

## Wounding of the Culture

Spore production was not stimulated by wounding the cultures (Table 5). The wounding spread the cultures of A. raphani and A. brassicae on a plate, but also, in both cases stimulated growth of aerial non-sporulating mycelium.

Table 4. Effect of a quantity of medium on yield of spores of A. brassicae and A. raphani grown on V-8 RB agar medium

| Fungus | Mean yield of spores/ml<br>15 ml of medium | <br>25 ml of medium |
|---|---|---|
| A. brassicae | 382,000 | 277,200 |
| A. raphani | 408,980 | 200,200 |

Table 5 Effect of wounding the culture on yield of spores of A. brassicae and A. raphani

| Days after Wounding | Mean yield of spores, spores/ml<br>A. brassicae<br>Control | <br><br>Wounded | <br>A. raphani<br>Control | <br><br>Wounded |
|---|---|---|---|---|
| 0 | 105 | 93 | 40 | 45 |
| 1 | 115 | 100 | 60 | 51 |
| 2 | 120 | 123 | 78 | 60 |
| 3 | 153 | 150 | 90 | 100 |
| 4 | 192 | 180 | 120 | 120 |
| 5 | 210 | 190 | 150 | 130 |
| 6 | 230 | 185 | 180 | 135 |
| 7 | 250 | 180 | 210 | 140 |
| 8 | 253 | 182 | 215 | 141 |

### Age of Culture

The highest yield of spores of *A. brassicae* and *A. raphani* was reached in 15-day-old and 12-day-old cultures, respectively (Figure 3). In both fungi, spore production was initiated after approximately 4 days, and rose linearly and rapidly till maximum spore production occurred. When maximum spore production was reached the fungus remained in a stationary phase. Spore production very closely resembles the classical lag-log-stationary normal growth curve.

## The Effect of *A. raphani* and *A. brassicae* on Yield and Quality of Rapeseed

Black spot of rapeseed caused Span and Zephyr to mature 3 to 15 days earlier than healthy plants. The more severe the disease the earlier the maturity.

### Analyses of Variances

### Effect of Black Spot on Yield of Rapeseed

a) Bulk-harvested material

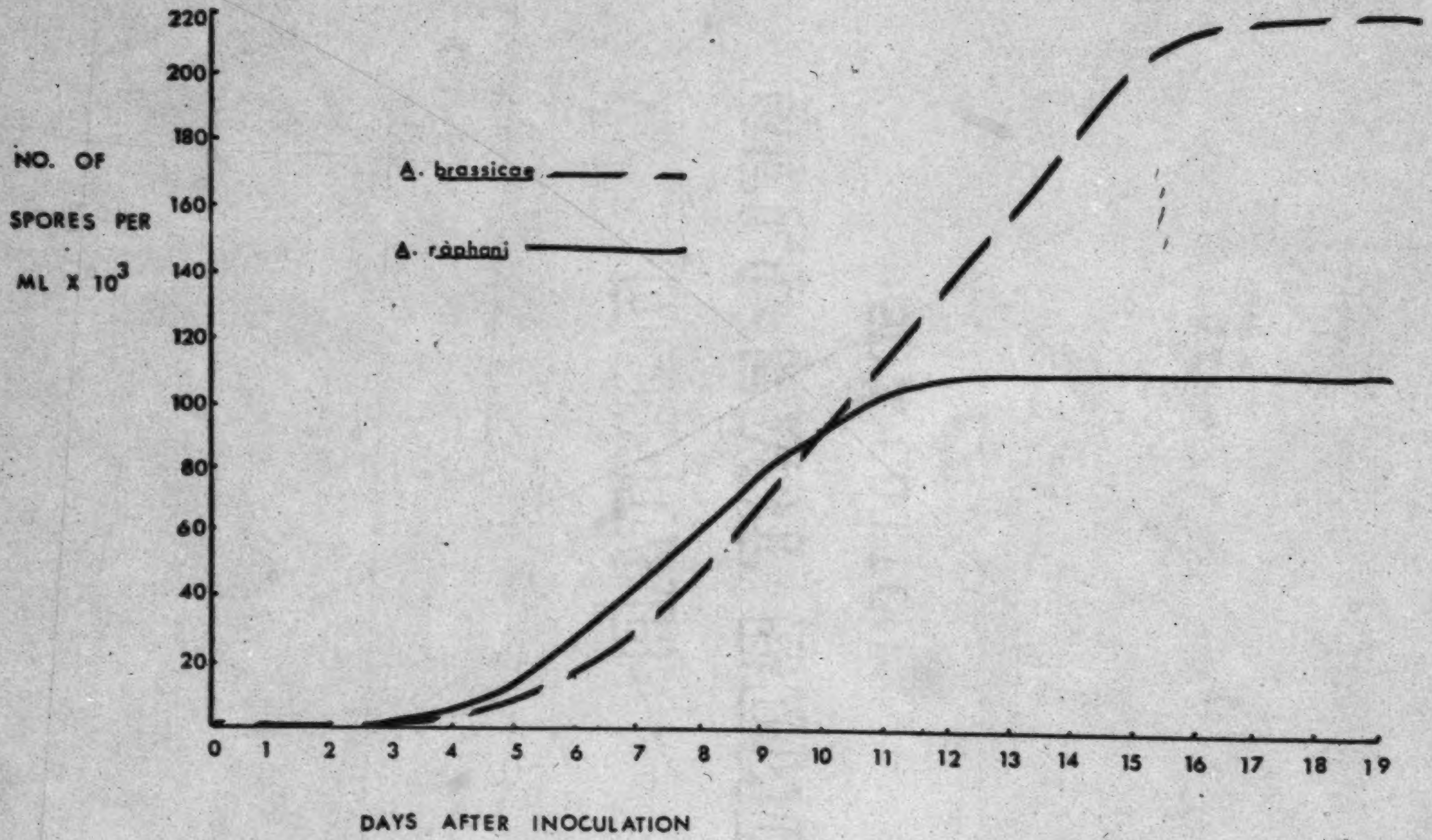


Fig. 3 Yield of spores of *Alternaria* brassicae and *A. raphani* cultures over a 19-day growing period.

Table 6. Effect of _A. raphani_, _A. brassicae_, and a combination of these _Alternaria_ spp. on yield of rapeseed of bulk-harvested material

| | Mean yield of rapeseed, g | | | |
|---|---|---|---|---|
| | | Inoculated with | | |
| Variety | Control | _A. raphani_ | _A. brassicae_ | Both spp. |
| Span | 192.6a* | 142.3ab | 108.5b | 112.3b |
| Zephyr | 153.8a | 107.6b | 108.8b | 116.5b |

*means within each variety followed by the same letter are not significantly different (LSD at the 5% level).

Black spot disease caused by _A. raphani_, _A. brassicae_ and a combination of both species reduced the yield of Span and Zephyr significantly, except for Span inoculated with _A. raphani_.

_A. brassicae_ reduced the yield of Span by 44% and that of Zephyr by 29%, while _A. raphani_ reduced the yield of Zephyr by 30%. A combination of both species reduced the yields by 42% and 24% for Span and Zephyr, respectively.

b) Disease-ranked material

Table 7. Effect of A. raphani, A. brassicae, and a combination of these species on the yield of rapeseed of disease-ranked material

| | | Mean yield of rapeseed, g | | |
|---|---|---|---|---|
| | | Inoculated with | | |
| Variety | Disease-ranked | A. raphani | A. brassicae | Both spp. |
| Span | Control | 22.4a* | 32.5a | 26.1a |
| | Slight | 22.8a | 29.3ab | 19.7b |
| | Moderate | 23.8a | 23.7b | 22.5ab |
| | Severe | 13.1b | 12.0c | 7.9c |
| Zephyr | Control | 30.9a | 26.0a | 29.4a |
| | Slight | 26.4a | 25.7a | 30.0a |
| | Moderate | 25.5a | 22.4a | 23.3b |
| | Severe | 20.5b | 15.0b | 17.0c |

*means within each variety and fungal inoculation group followed by the same letter are not significantly different (LSD at the 5% level)

In all instances, there were significant reductions in yield of rapeseed plants that were severely diseased (Table 7). Significant reductions in yield did not occur consistently in any other category of disease-ranked material of either variety. In the severely diseased material, A. raphani caused a yield reduction of 42% in Span and 34% in Zephyr; A. brassi-

*cae* caused a yield reduction of 63% in Span and 42% in Zephyr; a combination of both *Alternaria* spp. caused yield reductions of 70% and 41% in Span and Zephyr, respectively.

## Effect of Black Spot on Oil Content of Rapeseed

### a) Bulk-harvested Material

Table 8. Effect of *A. raphani*, *A. brassicae*, and a combination of these *Alternaria* spp. on oil content of rapeseed of bulk-harvested material

| | Mean oil content of rapeseed, % | | | |
|---|---|---|---|---|
| | | Inoculated with | | |
| Variety | Control | *A. raphani* | *A. brassicae* | Both spp. |
| Span | 37.2a | 36.1a | 36.4a | 36.7a |
| Zephyr | 35.8a* | 37.5a | 37.1a | 36.2b |

*means within each variety followed by the same letter are not significantly different (LSD at the 5% level)

The oil content of Zephyr was increased significantly by *A. raphani* and by *A. brassicae*, while the oil content of Span was not significantly affected by the disease (Table 8). *A. raphani* increased the oil content of Zephyr by 4.7%, while *A. brassicae* increased it by 3.6%.

b) Disease-ranked Material

Table 9. Effect of A. raphani, A. brassicae, and a combination of these species on the oil content of rapeseed of disease-ranked material

| | | Mean oil content of rapeseed, %<br>Inoculated with | | |
|---|---|---|---|---|
| Variety | Disease-rank | A. raphani | A. brassicae | Both spp. |
| Span | Control | 36.6a* | 26.9a | 37.1a |
| | Slight | 35.3b | 35.7b | 26.9a |
| | Moderate | 35.1b | 35.2b | 35.8a |
| | Severe | 35.3b | 35.1b | 33.6b |
| Zephyr | Control | 36.9a | 34.8a | 34.7a |
| | Slight | 36.7a | 33.3a | 34.4a |
| | Moderate | 36.1a | 33.4a | 34.9a |
| | Severe | 36.2a | 34.0a | 34.3a |

*means within each variety and fungal inoculation group followed by the same letter are not significantly different (LSD at the 5% level)

Significant reductions in the oil content of Span occurred in most of the plants affected with black spot disease, while no significant change in oil content occurred in Zephyr in any of the disease-ranked material (Table 9). In the severely diseased plants of Span the reductions in oil content caused by the *Alternaria* spp. were as follows: *A. raphani* -

3.6%, A. brassicae - 4.9%, and a combination of these species - 9.5%.

## Effect of Black Spot on Protein Content of Rapeseed

### a) Bulk-harvested Material

Table 10. Effect of A. raphani, A. brassicae, and a combination of these species on the protein content of rapeseed of bulk-harvested material

| | Mean protein content of the seed, % | | | |
|---|---|---|---|---|
| | | Inoculated with | | |
| Variety | Control | A. raphani | A. brassicae | Both spp. |
| Span | 25.9a* | 25.2b | 24.4c | 24.4c |
| Zephyr | 26.5a | 25.5b | 25.4b | 26.0ab |

*means within each variety followed by the same letter are not significantly different (LSD at the 5% level)

Black spot disease caused by A. raphani, A. brassicae, and a combination of A. brassicae and A. raphani, reduced the protein content of the seed of Span and Zephyr significantly, except for Zephyr infected with the combination of both spp. (Table 10).

A. raphani reduced the protein content of Span and Zephyr by 2.8% and 3.8%, respectively. A. brassicae reduced it by 5.8% and 4.2%, respectively; a combination of these species reduced the protein content of Span by 5.8%.

### b) Disease-ranked Material

Table 11. Effect of A. raphani, A. brassicae, and a combination of these species on the protein content of rapeseed of disease-ranked material

| | | Mean protein content of the seed, % Inoculated with | | |
|---|---|---|---|---|
| Variety | Disease-rank | A. raphani | A. brassicae | Both spp. |
| Span | Control | 25.1a* | 25.5a | 24.2a |
| | Slight | 24.6a | 24.9b | 24.4a |
| | Moderate | 23.9a | 24.8b | 24.3a |
| | Severe | 24.1a | 23.6c | 23.3b |
| Zephyr | Control | 24.2a | 24.7a | 25.3a |
| | Slight | 23.9a | 24.5a | 24.4b |
| | Moderate | 24.5a | 24.7a | 24.5b |
| | Severe | 24.3a | 25.1a | 25.5a |

*means within each variety and fungal inoculation group followed by the same letter are not significantly different (LSD at the 5% level)

The protein content of the seed of Span was significantly reduced in plants ranked severe and inoculated with A. brassicae and a combination of both species, while for Zephyr only the plants ranked slight and moderate inoculated with a combination of both species had their protein content reduced significantly (Table 11). In the severely diseased plants of Span the reductions in protein content caused by A. brassicae and a combination of both species were 7.5% and 3.8%, respectively.

## Effect of Black Spot on Protein Content of the Meal

### a) Bulk-harvested Material

Table 12. Effect of *A. raphani*, *A. brassicae*, and a combination of these species on the protein content of rapeseed meal of bulk-harvested material

| | Mean protein content of the meal, % | | | |
|---|---|---|---|---|
| | | Inoculated with | | |
| Variety | Control | *A. raphani* | *A. brassicae* | Both spp. |
| Span | 41.2a* | 39.5b | 38.4b | 38.5b |
| Zephyr | 41.3a | 40.8a | 40.4a | 40.7a |

*means within each variety followed by the same letter are not significantly different (LSD at the 5% level)

Significant reductions in the protein content of the meal of Span occurred in all the material having the black spot disease while the protein content of meal of Zephyr was not affected by the disease (Table 12). *A. raphani* reduced the protein content of the meal of Span by 4.2%; and a combination of these species reduced it by 6.6%.

b) Disease-ranked Material

Table 13. Effect of *A. raphani*, *A. brassicae*, and a combination of these species on protein content of rapeseed meal of disease-ranked material

| | | Mean protein content of the meal, % Inoculated with | | |
|---|---|---|---|---|
| Variety | Disease-rank | *A. raphani* | *A. brassicae* | Both spp. |
| Span | Control | 38.8a* | 40.5a | 38.0a |
| | Slight | 37.9a | 38.8b | 38.6a |
| | Moderate | 26.8b | 38.2b | 37.8a |
| | Severe | 37.3b | 36.4c | 35.2b |
| Zephyr | Control | 38.3a | 38.0a | 38.7a |
| | Slight | 37.8a | 36.7a | 37.7a |
| | Moderate | 38.5a | 37.1a | 37.3b |
| | Severe | 38.2a | 38.0a | 38.8a |

* means within each variety and fungal inoculation followed by the same letter are not significantly different (LSD at the 5% level)

The protein content of the meal of Span plants severely diseased was reduced significantly by *A. raphani*, *A. brassicae*, and a combination of these species, while for

Zephyr, the only significant reduction in protein content was in plants infected with both species ranked moderate for disease (Table 13). In the severely diseased plants of Span the reductions in protein content of the meal by *A. raphani*, *A. brassicae*, and a combination of both species were 3.9%, 10.2%, and 7.4%, respectively.

## Effect of Black Spot on One Thousand Kernel (Kwt) Weight of Rapeseed

### a) Bulk-harvested Material

Table 14. Effect of *A. raphani*, *A. brassicae*, and a combination of the species on 1000 Kwt of rapeseed of bulk-harvested material

| | Mean 1000 Kwt of rapeseed, g Inoculated with | | | |
|---|---|---|---|---|
| Variety | Control | *A. raphani* | *A. brassicae* | Both spp. |
| Span | 3.20a* | 2.70b | 2.41c | 2.46bc |
| Zephyr | 3.14b | 3.43a | 3.19b | 2.09b |

*means within each variety followed by the same letter are not significantly different (LSD at the 5% level)

Black spot disease caused by *A. raphani*, *A. brassicae*, and a combination of both species reduced the 1000 Kwt of Span significantly, while black spot disease caused by _A. raphani_ increased the 1000 Kwt of Zephyr significantly (Table 14). This resulted in reductions of 1000 Kwt of Span of 15.7%, 24.7%, and 23.2%, infected with _A. raphani_, _A. brassicae_, and a combination of both species, respectively. In Zephyr infected with _A. raphani_ there was an increase in 1000 Kwt of 9.2%.

b) Disease-ranked material

Table 15. Effect of _A. raphani_, _A. brassicae_, and a combination of these species on 1000 Kwt of rapeseed of disease-ranked material

| | | Mean 1000 Kwt of rapeseed, g Inoculated with | | |
|---|---|---|---|---|
| Variety | Disease-rank | _A. raphani_ | _A. brassicae_ | Both spp. |
| Span | Control | 2.66a* | 2.58a | 2.90a |
| | Slight | 2.51a | 2.49a | 2.71a |
| | Moderate | 2.51a | 1.99b | 2.55a |
| | Severe | 2.32a | 1.84b | 1.90b |
| Zephyr | Control | 3.32a | 3.37a | 3.56a |
| | Slight | 3.49a | 3.48a | 3.32a |
| | Moderate | 3.36a | 3.46a | 3.43a |
| | Severe | 2.93b | 2.75b | 3.00b |

*means within each variety and fungal inoculation group followed by the same letter are not significantly different

(LSD at the 5% level)

In all instances, except Span inoculated with *A. raphani*, the 1000 Kwt of seed was reduced significantly in the severely diseased plants (Table 15). *A. raphani* reduced the 1000 Kwt of Zephyr by 11.8%; *A. brassicae* reduced it in Span and Zephyr by 24.9% and 18.4%, respectively; a combination of these species reduced it by 34.5% and 15.9%, respectively.

## Effect of Black Spot on Seed Volume of Rapeseed

### a) Bulk-harvested Material

Table 16. Effect of *A. raphani*, *A. brassicae*, and a combination of both species on seed volume of rapeseed of bulk-harvested material

| | Mean volume of rapeseed, cc Inoculated with | | | |
|---|---|---|---|---|
| Variety | Control | *A. raphani* | *A. brassicae* | Both spp. |
| Span | 2.85a* | 2.30b | 2.07c | 2.15bc |
| Zephyr | 2.95a | 3.20a | 2.97a | 2.90a |

*means within each variety followed by the same letter are not significantly different (LSD at the 5% level)

The seed volume of Span was reduced significantly by *A. raphani*, *A. brassicae*, and a combination of both species (Table 16). The 1000 seed volume of Zephyr was not affected by the disease. The decrease in seed volume of Span was 19.3% in plants infected by *A. raphani*, 27.0% in plants infected by *A. brassicae*, and 24.6% in plants infected by a combination of both species.

b) Disease-ranked material

Table 17. Effect of *A. raphani*, *A. brassicae*, and a combination of both species on seed volume of rapeseed of disease-ranked material

| | | Mean volume of rapeseed, cc Inoculated with | | |
|---|---|---|---|---|
| Variety | Disease Rank | *A. raphani* | *A. brassicae* | Both spp. |
| Span | Control | 2.27a* | 2.25a | 2.57a |
| | Slight | 2.15a | 2.10a | 2.30a |
| | Moderate | 2.17a | 1.67b | 2.22ab |
| | Severe | 1.87b | 1.55b | 1.70b |
| Zephyr | Control | 3.17a | 3.12a | 3.37a |
| | Slight | 3.20a | 3.22a | 3.05b |
| | Moderate | 2.90b | 3.27a | 3.07b |
| | Severe | 2.67c | 2.57b | 2.70c |

*means within each variety and fungal inoculation group followed by the same letter are not significantly different

(LSD at the 5% level)

The seed volume was significantly reduced in the severely diseased plants of Span and Zephyr inoculated with _A_. _raphani_, _A_. _brassicae_, and both species (Table 17). Significant reductions in yield did not occur consistently in the other disease ratings. The reductions in seed volume of severely diseased plants of Span and Zephyr inoculated with _A_. _raphani_ were 17.7% and 15.8%, respectively; infected with _A_. _brassicae_ were 39.3% and 17.7%, respectively; and a combination of both species were 33.9% and 19.9%, respectively.

## Effect of Black Spot on Seed Density of Rapeseed

a) Bulk-harvested Material

Table 18. Effect of _A_. _raphani_, _A_. _brassicae_, and a combination of both species on seed density of rapeseed of bulk-ranked material

| | Mean seed density of rapeseed, g/cc | | | |
|---|---|---|---|---|
| | | Inoculated with | | |
| Variety | Control | _A_. _raphani_ | _A_. _brassicae_ | Both spp. |
| Span | 1.12b* | 1.17a | 1.16a | 1.14ab |
| Zephyr | 1.06a | 1.07a | 1.07a | 1.07a |

*means within each variety followed by the same letter are not significantly different (LSD at the 5% level)

The seed density of Span was increased significantly in plants inoculated with _A_. _raphani_ and _A_. _brassicae_ while in all other instances there were no significant changes (Table 18).

b) Disease-ranked Material

Table 19 Effect of _A_. _raphani_, _A_. _brassicae_, and a combination of both species on seed density of rapeseed of disease-ranked material

| | | Mean seed density of rapeseed, g/cc Inoculated with | | |
|---|---|---|---|---|
| Variety | Disease-rank | _A_. _raphani_ | _A_. _brassicae_ | Both spp. |
| Span | Control | 1.19a* | 1.15a | 1.14a |
| | Slight | 1.17a | 1.18a | 1.18a |
| | Moderate | 1.15a | 1.19a | 1.15a |
| | Severe | 1.24a | 1.19a | 1.16a |
| Zephyr | Control | 1.04a | 1.09a | 1.05a |
| | Slight | 1.09a | 1.08a | 1.09a |
| | Moderate | 1.16a | 1.06a | 1.12a |
| | Severe | 1.09a | 1.07a | 1.11a |

*means within each variety and fungal inoculation group followed by the same letter are not significantly different (LSD at the 5% level)

The black spot disease did not have a significant effect on the seed density of rapeseed in the disease-ranked material (Table 19).

## Effect of Black Spot on the Fatty Acids of the Oil of Rapeseed

### a) Bulk-harvested Material

Table 20. Effect of *A. raphani*, *A. brassicae*, and a combination of these species on the palmitic, oleic, linoleic, and linolenic acid of the oil of rapeseed of bulk-harvested material

| | | Mean fatty acid content of the seed,% | | | |
|---|---|---|---|---|---|
| | | | Inoculated with | | |
| Variety | Fatty Acid | Control | *A. raphani* | *A. brassicae* | Both spp. |
| Span | Palmitic | 2.8a* | 3.2a | 3.9a | 3.8a |
| | Oleic | 64.8a | 65.5a | 63.0a | 63.7a |
| | Linoleic | 23.9a | 21.8a | 23.1a | 23.5a |
| | Linolenic | 8.1a | 8.6a | 9.9a | 7.7a |
| Zephyr | Palmitic | 3.8a | 2.8a | 4.1a | 3.8a |
| | Oleic | 68.9a | 74.2a | 67.5a | 69.5a |
| | Linoleic | 22.9a | 17.7b | 23.0a | 21.3a |
| | Linolenic | 4.4a | 5.1a | 5.3a | 5.3a |

*means within each variety and each fatty acid followed by the same letter are not significantly different (LSD at the 5% level)

The linoleic acid of the oil of Zephyr was significantly reduced by *A. raphani* (Table 20). The disease had no effect on the fatty acid content in all of the other instances.

b) Disease-ranked Material

Table 21. Effect of *A. brassicae* on the palmitic, oleic, linoleic, and linolenic acid of the oil of rapeseed of disease-ranked material

| | | Mean fatty acid content of the seed, % Inoculated with *A. brassicae* | | | |
|---|---|---|---|---|---|
| Variety | Disease-rank | Palmitic | Oleic | Linoleic | Linolenic |
| Span | Control | 3.74b* | 60.6a | 22.3ab | 11.2a |
| | Slight | 3.57b | 60.2a | 21.8b | 11.7a |
| | Moderate | 3.99a | 58.4a | 23.2a | 11.2a |
| | Severe | 3.87ab | 61.0a | 23.0a | 10.7a |
| Zephyr | Control | 4.95a | 64.6a | 20.9a | 9.51a |
| | Slight | 5.14a | 65.9a | 20.4a | 9.30a |
| | Moderate | 4.80a | 64.8a | 21.1a | 9.30a |
| | Severe | 5.20a | 63.9a | 21.4a | 9.50a |

*means within each variety and each fatty acid group followed by the same letter are not significantly different (LSD at the 5% level)

The palmitic and linoleic acid content of the oil of Span was significantly increased in the moderately diseased material (Table 21).

In all other instances there was not significant changes in the fatty acid content of the oil.

## Effect of Black Spot on the Glucosinolates Present in the Rapeseed Meal

a) Bulk-harvested Material

Table 22. Effect of _A_. _raphani_, _A_. _brassicae_, and a combination of these species on the BI, PI, MeSB, PhE and OZT content of rapeseed meal of bulk-harvested material

| | | Mean isothiocyanate and OZT of the meal, mg/g Inoculated with | | | |
|---|---|---|---|---|---|
| Variety | Isothiocyanates & OZT | Control | _A. raphani_ | _A. brassicae_ | Both spp. |
| Span | BI | 2.00a* | 1.87a | 2.07a | 1.95a |
| | PI | 2.12a | 2.15a | 1.57a | 2.12a |
| | MeSB | 0.32a | 0.35a | 0.10a | 0.27a |
| | PhE | 0.77a | 1.00a | 0.42a | 0.65a |
| | OZT | 0.65a | 0.60a | 0.77a | 1.00a |
| Zephyr | BI | 0.91a | 1.03a | 0.85a | 0.71a |
| | PI | 0.11a | 0.13a | 0.12a | 0.09a |
| | MeSB | 0.05a | 0.07a | 0.06a | 0.05a |
| | PhE | 0.07a | 0.07a | 0.14a | 0.06a |
| | OZT | 8.67a | 8.99a | 7.99a | 7.22a |

*means within each variety and each glcosinolate group followed by the same letter are not significantly different (LSD at the 5% level)

The isothiocyanates and OZT were not significantly affected by black spot in the bulked material (Table 22).

b) Disease-ranked Material

Table 23. Effect of A. brassicae on the BI, PI, MeSB, PhE, and OZT content of rapeseed meal of disease-ranked material

| | | Mean isothiocyanate and OZT of the meal, mg/g<br>Inoculated with A. brassicae | | | | |
|---|---|---|---|---|---|---|
| Variety | Disease-rank | BI | PI | MeSB | PhE | OZT |
| Span | Control | 2.47a* | 2.34a | 0.25a | 0.46a | 2.45a |
| | Slight | 2.37a | 2.61a | 0.24a | 0.52a | 2.65a |
| | Moderate | 1.54b | 1.73b | 0.23a | 0.32a | 2.93a |
| | Severe | 1.50b | 1.80b | 0.28a | 0.49a | 2.82a |
| Zephyr | Control | 1.17a | 0.29a | 0.13a | 0.08a | 8.80a |
| | Slight | 1.16a | 0.22a | 0.17a | 0.08a | 7.96a |
| | Moderate | 1.40a | 0.28a | 0.21a | 0.08a | 7.54a |
| | Severe | 1.27a | 0.31a | 0.25a | 0.11a | 7.86a |

*means within each variety and glucosinolate group followed by the same are not significantly different (LSD at the 5% level)

The BI and PI content of the meal of Span was significantly reduced in the moderately and severely diseased material (Table 23). In all other instances there was no significant effect of black spot on the isothiocyanates and OZT of the meal.

## Correlations

a) Bulk-harvested material

Table 24. Simple correlation coefficients between yield, oil and protein content of the seed, protein content of the meal, 1000 Kwt, 1000 seed volume, and seed density of bulk-harvested Span

(Values for N=14)

| | Yield | Oil | Prot S | Prot M | 1000 Kwt | Seed Volume | Seed Density |
|---|---|---|---|---|---|---|---|
| Oil | .18 | - | | | | | |
| Prot S | .76** | .19 | - | | | | |
| Prot M | .73** | .49 | .95** | - | | | |
| 1000 Kwt | .86** | .22 | .76** | .75** | - | | |
| Seed Volume | .82** | .28 | .73** | .74** | .98** | - | |
| Seed Density | -.10 | -.39 | -.12 | -.21 | -.33 | -.50 | - |

*significant at P=0.05, **significant at P=0.01

Significant positive correlations occurred between all quality and yield components, except oil content of the seed and seed density which did not correlate significantly with each other or any other component (Table 24).

Table 25 Simple correlation coefficients between yield, oil content of the seed, and the fatty acids present in the oil of bulk-harvested Span (Values for N=14)

| | Yield | Oil | Palmitic | Oleic | Linoleic | Linolenic |
|---|---|---|---|---|---|---|
| Oil | .18 | - | | | | |
| Palmitic | -.53* | -.05 | - | | | |
| Oleic | .21 | .16 | -.23 | - | | |
| Linoleic | .08 | -.15 | -.06 | -.83** | - | |
| Linolenic | -.25 | -.09 | .18 | -.39 | -.02 | - |

*significant at P=0.05; significant at P=0.01

Significant negative correlations occurred between yield and palmitic acid, and between oleic and linoleic acid (Table 25).

Table 26. Simple correlation coefficients between yield, protein content of the seed and meal, and glucosinolates in the meal of bulk-harvested Span
(Values for N=14)

| | Yield | Prot S | Prot M | BI | PI | MeSB | PhE | OZT |
|---|---|---|---|---|---|---|---|---|
| Prot S | .76** | - | | | | | | |
| Prot M | .73** | .95** | - | | | | | |
| BI | .25 | .11 | .09 | - | | | | |
| PI | .44 | .32 | .22 | .59* | - | | | |
| MeSB | .26 | .27 | .11 | -.23 | .26 | - | | |
| PhE | .36 | .35 | .18 | .01 | .39 | .85** | - | |
| OZT | -.18 | -.33 | -.36 | -.20 | -.06 | .14 | .16 | - |

*significant at P=0.05; **significant at P=0.01

Significant positive correlations occurred between yield, protein content of the seed, and protein content of the meal, as well as between PI and BI and between MeSB and PhE (Table 26).

b) Disease-ranked Material

Table 27. Simple correlation coefficients between yield, oil and protein content of the seed, protein content of the meal, 1000 Kwt, 1000 seed volume and seed density of Span inoculated with *A. brassicae*.
(Upper values: data from control, N=10)
(Lower values: data from severe, N=10)

| | Yield | Oil | Prot S | Prot M | 1000 Kwt | Seed Volume | Seed Density |
|---|---|---|---|---|---|---|---|
| Oil | 0.15 | | | | | | |
| | 0.38 | | | | | | |
| Prot S | -0.28 | 0.08 | | | | | |
| | 0.39 | 0.37 | | | | | |
| Prot M | .16 | 0.57 | .86** | | | | |
| | 0.46 | 0.83** | .83** | | | | |
| 1000 Kwt | -0.23 | -0.47 | 0.54 | 0.20 | | | |
| | 0.55 | 0.05 | 0.32 | 0.22 | | | |
| Seed Volume | -0.02 | -0.34 | 0.48 | 0.23 | 0.90** | | |
| | 0.54 | 0.10 | 0.34 | 0.26 | 0.94** | | |
| Seed Density | -0.35 | -0.33 | 0.27 | 0.05 | 0.51 | 0.18 | |
| | 0.35 | 0.14 | 0.14 | 0.01 | 0.64* | 0.35 | - |

*significant at P=0.05; **significant at P=0.01

Significant positive correlations occurred between oil content of the seed and protein content of the meal, protein content of the seed and protein content of the meal, 1000 Kwt and seed volume, and between 1000 Kwt and seed density (Table 27). Twice as many significant correlations occurred in the severe group as in the control.

Table 28. Simple correlation coefficients between yield, oil content of the seed, and fatty acids present in the oil of Span inoculated with _A_. _brassicae_. (Upper values: data from control, N=10; lower values: data from severe, N=10)

| | Yield | Oil | Palmitic | Oleic | Linoleic | Linolenic |
|---|---|---|---|---|---|---|
| Oil | .15 | - | | | | |
| | .38 | - | | | | |
| Palmitic | -.19 | -.26 | - | | | |
| | -.68* | -.56 | - | | | |
| Oleic | .17 | .21 | -.55 | - | | |
| | .24 | .41 | -.14 | - | | |
| Linoleic | -.71** | .34 | -.18 | .40 | - | |
| | -.60* | -.49 | .70* | -.18 | - | |
| Linolenic | -.37 | -.11 | .73** | -.82** | .36 | - |
| | .27 | -.28 | -.06 | -.79** | .07 | - |

*significant at P=0.05; **significant at P=0.01

Significant positive correlations occurred between palmitic and linoleic acid and between palmitic and linolenic acid, while both palmitic and linoleic acid were significantly negatively correlated with yield (Table 28). There was also a negative correlation in both the control and severe groups between oleic and linolenic acid.

Table 29. Simple correlation coefficients between yield, protein content of the seed and meal, and glucosinolates in the meal of Span inoculated with *A. brassicae*

(Upper values: data from control, N=10;
lower values: data from severe, N=10)

| | Yield | Prot S | Prot M | BI | PI | MeSB | PhE | OZT |
|---|---|---|---|---|---|---|---|---|
| Prot S | -.28 | - | | | | | | |
| | .39 | - | | | | | | |
| Prot M | -.16 | .86** | - | | | | | |
| | .47 | .83** | - | | | | | |
| BI | .42 | -.04 | -.15 | - | | | | |
| | -.06 | .11 | -.06 | - | | | | |
| PI | .32 | -.05 | .12 | .48 | - | | | |
| | -.14 | .08 | -.06 | .89** | - | | | |
| MeSB | -.38 | .04 | .01 | .11 | -.15 | - | | |
| | .67* | .18 | .38 | -.26 | -.46 | - | | |
| PhE | -.33 | .40 | .17 | -.02 | -.71** | .37 | - | |
| | -.31 | .15 | .20 | -.27 | -.60* | .34 | - | |
| OZT | -.09 | -.33 | -.29 | -.02 | -.48 | .25 | .49 | - |
| | -.67* | -.22 | -.28 | -.28 | -.12 | -.25 | .40 | - |

*significant at P=0.05; **significant at P=.01

Significant positive correlations occurred between protein of the seed and protein of the meal, BI and PI, and yield and MeSB while significant negative correlation occurred between yield and OZT and PI and PhE (Table 29). More than twice as many significant correlations occurred in the severe group as in the control group.

## Effect of Black Spot on Floret Abortion and Floret Numbers

Black spot lesions appeared on the leaves and stems 2 days after inoculation. They developed rapidly with maximum lesioning in about 7 days after inoculation. *A. raphani* and *A. brassicae* infection increased the flowering period by over a week compared to the control.

Table 30. Effect of *A. brassicae* and *A. raphani* on the numbers of florets aborting in the rapeseed varieties, Span and Zephyr.

| | Number of Mean Floret Abortion of Plants, % Inoculated with | | |
|---|---|---|---|
| Variety | Control | *A. raphani* | *A. brassicae* |
| Span | 21.8b* | 48.9a | 54.9a |
| Zephyr | 20.6b | 37.8a | 39.6a |

*means within each variety followed by the same letter are not significantly different (LSD at the 5% level)

A significant increase in per cent aborted florets occurred on Span and Zephyr as a result of black spot caused by *A. brassicae* and *A. raphani*. (Table 30).

Table 31. Effect of infection by _A_. _brassicae_ and _A_. _raphani_ on floret numbers in rapeseed varieties, Span and Zephyr.

| | Mean Total Number of Florets of Plants Inoculated with | | |
|---|---|---|---|
| Variety | Control | _A_. _raphani_ | _A_. _brassicae_ |
| Span | 116.8b* | 259.4a | 202.8a |
| Zephyr | 98.2a | 127.1a | 107.9a |

*means within each variety followed by the same letter are not significantly different (LSD at the 5% level)

A significant increase in total number of florets occurred on Span infected with _A_. _brassicae_ or _A_. _raphani_ (Table 31). Black spot had no effect on the total number of florets on Zephyr.

## Toxic Metabolites

### Germinating Seeds

Seedlings germinated on distilled water, V-8 liquid medium, on the filtrate of *A. humicola* were erect, approximately 40-50 mm tall, and the cotyledons were uniformly green.

Seedlings germinated on filtrates of *A. brassicae* or *A. raphani* were noticeably stunted (20-40 mm), and in many instances distinct chlorotic spots occurred on cotyledons. There was little observable difference in the appearance of cotyledons from autoclaved and non-autoclaved filtrates. The most significant effect of these filtrates was on the roots. They were poorly developed with few root hairs, and were brown in color as compared to the creamy white coloration of the roots in other treatments.

### Detached Leaves

In distilled water and in V-8 liquid medium (autoclaved and non-autoclaved) the leaves remained turgid and uniformly green during the duration of the experiment.

On leaves artifically inoculated with *A. brassicae* spores, after 7 days typical *Alternaria* leaf lesions had developed (Plate 1.A). The lesions had the brown necrotic center surrounded by one or more chlorotic halos. The necrotic center was totally collapsed with each surrounding zone being less collapsed until the relatively healthy, turgid tissue was reached (Main, 1971). In the necrotic centers and some chlorotic zones, darker brown necrotic veins were seen.

In the autoclaved filtrate of A. humicola the leaves retained their turgidity and, although the coloration was uniform, it was a slightly lighter shade of green (Plate 1. B).

In the non-autoclaved filtrate of A. humicola there was a more distinct chlorosis in less than half of the leaves beginning on the sixth day (Plate 2. E). At this time, discrete light green totally collapsed areas appeared in the more chlorotic leaves. These central areas were surrounded by a less collapsed chlorotic halo. The leaf tissue surrounding the halo was still turgid.

The other leaves in the non- autoclaved filtrate of A. humicola remained turgid and green.

In the autoclaved filtrates of A. raphani the leaves developed dry, collapsed, light green areas surrounded by a chlorotic zone within five days (Plate 1. C). Veins in the chlorotic zone were brown in color. At 7 days, the cells in the light green areas had turned brown, and developed a series of light yellow halos.

In the non-autoclaved filtrates of A. raphani, the same symptoms appeared as in the autoclaved filtrates except that they occurred in about half the period of time, and were expressed more intensely at the end of 7 days (Plate 2. F).

In autoclaved filtrates of A. brassicae, the discrete light green, dry areas surrounded by a chlorotic zone appeared in about 36 hours (Plate 1. D). By 5 days, these light green areas had collapsed completely, turned brown in color, and developed a definite series of chlorotic halos. The whole leaf was chlorotic and the veins were dark brown in color in these areas.

In non-autoclaved filtrates of A. brassicae, the above symptoms appeared in about half the period of time (Plate 2. G). In addition, approximately half the leaves had wilted completely in three days. They were strongly chlorotic and were curled at the edges. Browning of the veins was obvious in the chlorotic areas.

Plate 1

Zephyr leaves after 7 days of the following treatments:

A) Inoculated with _A. brassicae_ spores; note that the small lesions have necrotic brown veins running through them;

B) An autoclaved filtered solution of _A. humicola;_

C) An autoclaved filtered solution of _A. raphani_;and

D) An autoclaved filtered solution of _A. brassicae_.

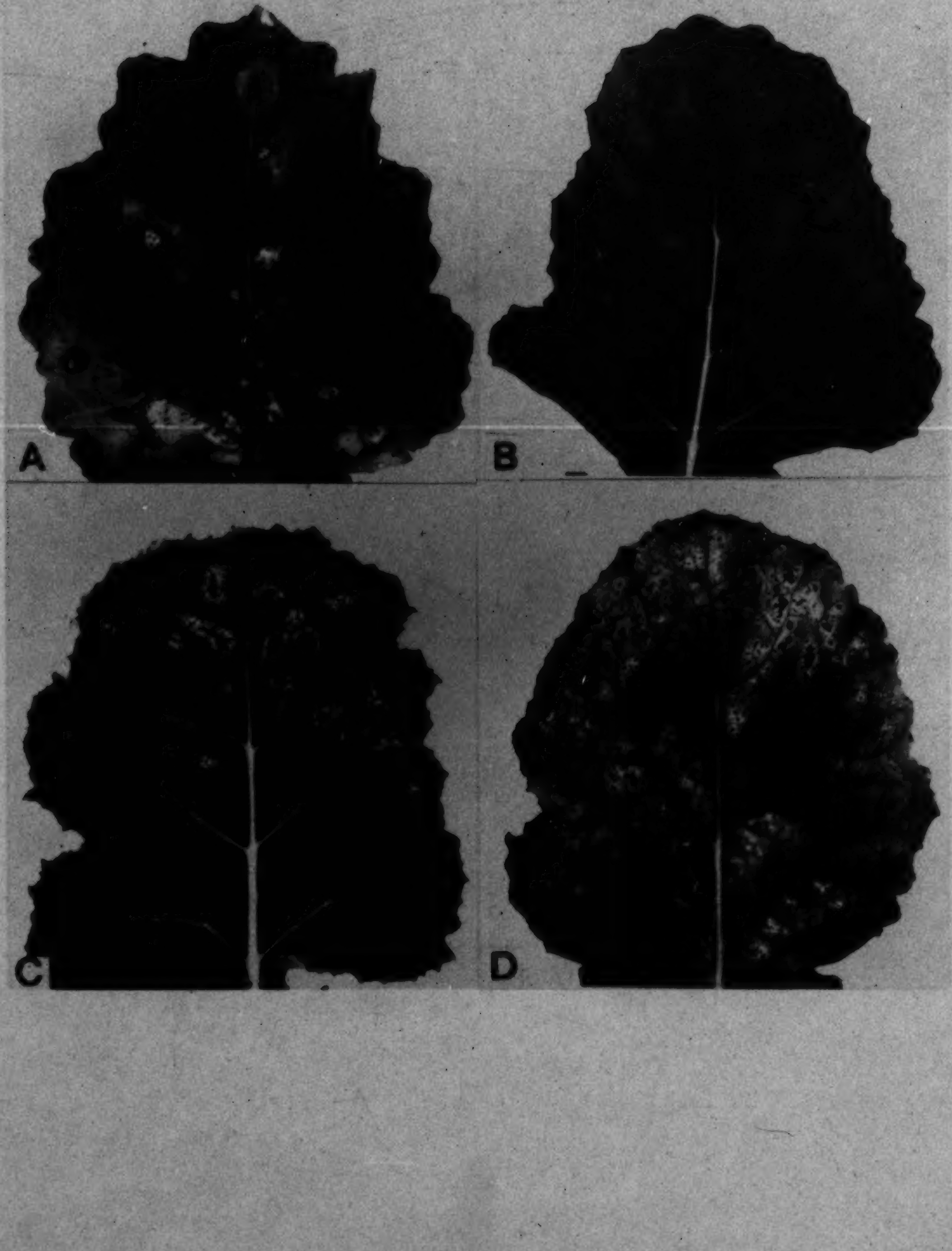

A
B
C
D

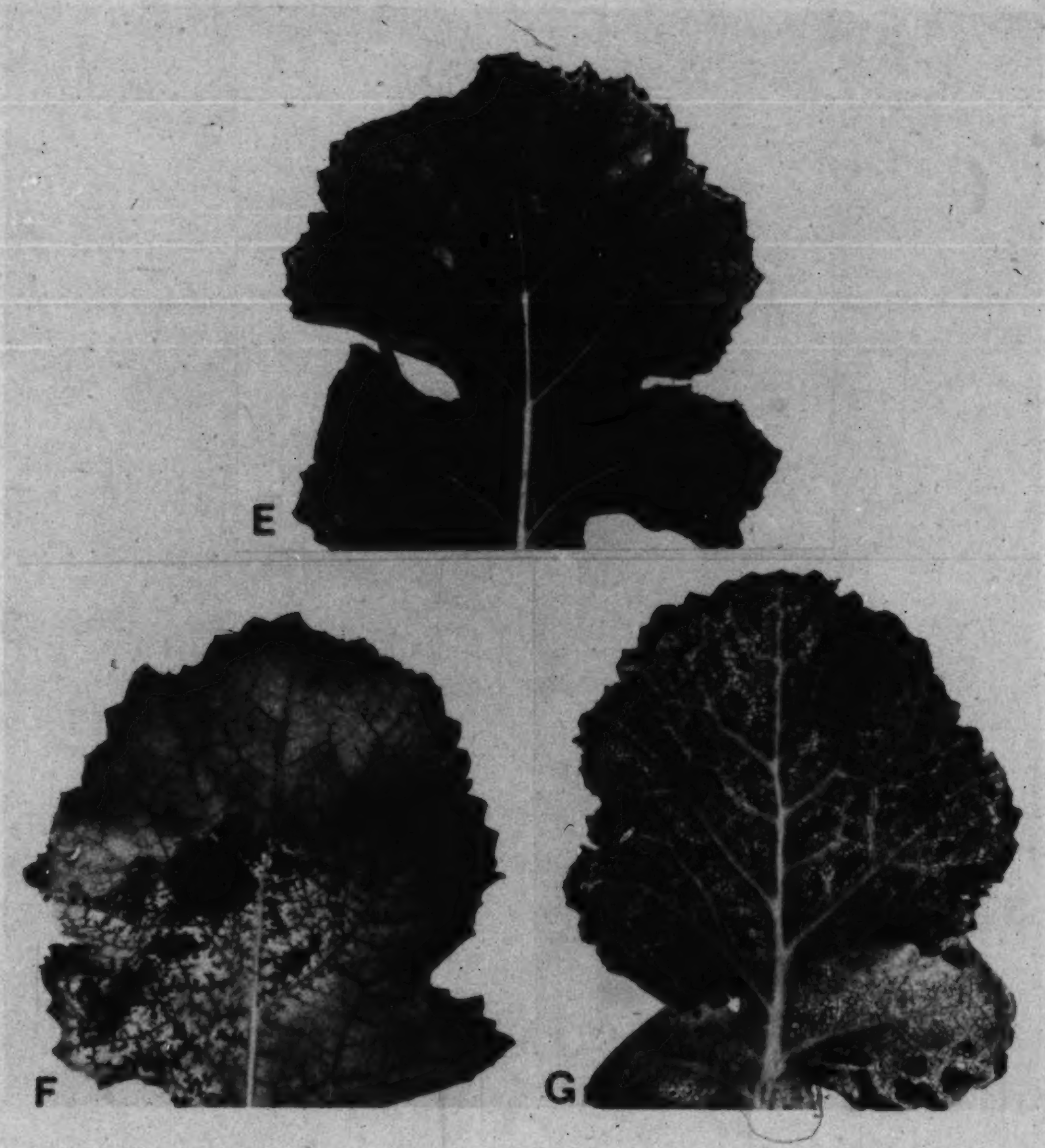
Plate 2

Zephyr leaves treated 7 days with, respectively, filtered solutions of:

E) _A_. _humicola_;

F) _A_. _raphani_; and

G) _A_. _brassicae_.


E
F
G

# DISCUSSION

The main objective of this investigation was to determine the effect of Black spot disease caused by A. raphani and A. brassicae on the yield and certain quality factors of rapeseed. In order to do so, a large, reliable supply of spores for inoculation had to be realized. Thus, the first part of the study consisted of growing the fungi in artificial culture under various conditions to produce a consistent, abundant supply of spores.

The factors tested to increase sporulation were nutrient agar media, seeding load, Rose Bengal concentration, temperature, light, quantity of agar, wounding of the culture, and age of culture.

The different nutrient agar media had marked effects on sporulation. Other researchers have also found this variation in sporulation due to media (Atkinson, 1950; McDonald, 1959; Taber, 1963; Changsri and Weber, 1963; and Ionnoidis and Main, 1973). V-8 juice agar with Rose Bengal plus streptomycin stimulated sporulation of A. raphani and A. brassicae to the greatest extent. McDonald (1959) and Ionnoidis and Main (1973) showed V-8 juice agar stimulated sporulation slightly. Rose Bengal, itself, has often been used in fungal isolation work, because of its ability to reduce bacterial and actinomycete development as well as reducing mycelial growth of fungi to some extent (Smith and Dawson, 1944). With the Alternaria species, A.

brassicae and A. raphani, the effect of Rose Bengal in different concentrations was very marked. The higher the Rose Bengal concentration, the slower was the colony growth of both species. The spores produced from these cultures differing in Rose Bengal concentration followed a skewed mono-model curve with its peak at 0.004% Rose Bengal. Therefore, at this concentration Rose Bengal inhibited mycelial growth while stimulating sporulation to the greatest extent.

The depth of medium in a Petri plate affected spore production, with a minimal amount (15 ml) producing the most spores. The V-8 RB medium had dried up considerably in 12 to 16 days and was starting to curl up at the margins. This increase in sporulation may be accounted for in that the medium was nearly completely exhausted causing a stress on the fungi increasing spore production. The other stress factor inherent with a minimal amount of medium was the greater concentration of fungal metabolite by-products.

When wounding of the colony to stimulate sporulation was tried, no increase in spore production occurred. This may have happened because the stress condition of the small amount of medium, its dehydration, and the inhibitory properties of Rose Bengal had already stimulated maximum spore production. In addition, in scarring the colony, new colonies were started from mycelial and spore debris. Thus, more individual colonies were present, which according to the experiment on seeding load would reduce spore yield. Other researchers who have utilized wounding to stimulate sporulation of Alternaria species have used different techniques (Ludwig et al., 1962; and Billotte, 1963). In their techniques the cultures were washed thoroughly after wounding to remove spore and mycelial debris, and then left exposed to the air for drying.

The effect of seeding load on spore production by A. brassicae has been commented on before by McDonald (1959). He found that on ADA, an antagonism existed between A. brassicae spores. This antagonism resulted in smaller colony size and low spore production as occurred in this study on V-8 RB. The greater the seeding load, the lower was the subsequent spore production, following a sigmoidal curve. Single spore cultures produced the greatest amount of spores on V-8 RB for both A. brassicae and A. raphani. The reason for this may be because, as McDonald (1959) suggests, an antagonistic relationship may exist between spores. Or, as Ludwig et al. (1962) states, that cultures of Alternaria solani tend to build-up an antisporulating agent. Single spore cultures, over the short 2 week growing period would not be capable of as rapid a build up of this antisporulating agent as would higher spore loads.

Optimum temperature and light conditions for spore production obtained in this study did not differ much from what other workers have found (Neergaard, 1945; Lukens, 1962; Changsri and Weber, 1963; Taber, 1963). The optimal temperature for sporulation of the working isolates of *A. raphani* and *A. brassicae* was 23°C. The optimal light regime was a diurnal light of 100-150 foot candles of incandescent light with 8 hours light and 16 hours of darkness. This amount and quality of light appeared optimal for two reasons. The first was, as indicated by Lukens (1962); a diurnal light was required for spore production of *Alternaria* spp. The second, as shown by Taber (1963), was that light strong in the red wavelengths stimulated conidial production by *A. raphani*.

The effect of age of culture for optimal sporulation was similar to previous reports (McDonald, 1959; and Taber, 1963).

The yield and certain quality factors of rapeseed were affected by the black spot disease. In general, it appears that *A. brassicae* was more pathogenic on rapeseed than *A. raphani* in 1972. The combination of both species, although in some cases causing greater damage than the single species, was usually not as pathogenic as the most pathogenic of the single species. Vanterpool (1963) found *A. brassicae* was favoured in wet years and *A. raphani* in dry years. This probably was the major reason *A. brassicae* was more pathogenic than *A. raphani* in 1972. This could also account for the reduced virulence of the inoculum of the combination of both species, since *A. brassicae* was half as concentrated in this inoculum.

Span (*B. campestris*) suffered greater damage due to black spot than Zephyr (*B. napus*). Both Husain and Thakur (1963) and Bhander and Maini (1965) found that in studies of resistance of oleiferous *Brassica* species to *Alternaria* blight; *B. napus* was resistant in some cases, but *B. campestris* was always susceptible to highly susceptible. The studies of Husain and Thakur (1963) and Bhander and Maini (1965) were carried out on only leaf evaluations of resistance. In the flowering stage of growth using lesion development as the criteria, Span and Zephyr appeared equally as susceptible to black spot; but in the earlier stages Zephyr was more resistant. Thus, this study at early stages agrees with their work; but from flowering on, *B. napus* did not display any outstanding resistance on leaves, stems or pods as suggested by those authors.

In most cases, the black spot disease reduced yield, 1000 Kwt and seed volume significantly, but did not have a consistent effect on seed density. It was found that reductions in 1000 Kwt and seed volume accounted for only approximately half of the total reductions in yield. Thus, some other components of yield of rapeseed were affected by the black spot disease. Other components of yield that could of been affected were number of pods per plant, number of seeds per pod and pod length. It was found that *A. raphani* and *A. brassicae* had drastically increased the number of florets aborting on the rapeseed varieties, Zephyr and Span, in comparison to non-inoculated controls. *A. raphani* and *A. brassicae* also, increased the period of flowering and the total number of florets produced by Span and Zephyr in the greenhouse. The effect of increasing total number of florets and the period of flowering could be similar to the effect of *A. alternata* on tobacco reported by Stavely and Slana (1973). They found *A. alternata* increased the leaf production of tobacco. Sackston (1950) found pasmo caused the most damage during the flowering stage in flax. The resulting yield reduction was not attibutable totally to reductions in seed size. Weber *et al*. (1966) working with soybeans, found that brown stem rot decreased yield to the greatest extent by decreasing seed number. The decrease in seed number was attributed to pod or seed abortion occurring during flowering. Louvet and Billotte (1964) implied that black spot of rapeseed caused floret abortion, when they stated that the rapeseed pods could be protected from black spot, caused by *A. brassicae*, by a fungicide treatment carried out only during flowering. The treatment would suppress spore-formation on the foliar ring-spots during this most sensitive stage of pod development. It appeared that floret abortion could be a major cause of yield reductions in black spot-diseased rapeseed varieties.

When yield and its components, 1000 Kwt and seed volume were correlated, consistent, significant, positive correlations were obtained. The seed volume was reduced to a greater extent by the disease than the 1000 Kwt. This would account for the disease increasing the seed density of Span in the bulk material, and the positive correlation obtained between 1000 Kwt. and seed density. There are few reports on the effect of diseases on yield of rapeseed, but Sackston (1950), Hartwig and Johnson (1953) and Zimmer and Zimmerman (1972) working with other oil crops, showed that diseases can significantly reduce the yield, 1000 Kwt., and seed volume of those crops.

The major quality factors of rapeseed were the oil and protein content of the seed. There was no significant change in the oil content of bulk-harvested Span, but there was a significant reduction in oil content in the disease-ranked material of Span. There was no consistent significant changes in oil content in Zephyr due to black spot. Thus, for oil content it appears that if there was a great enough reduction in yield, the oil content would be reduced, if the reduction was between 20 and 45 per cent, inconsistent results would be obtained.

Span and Zephyr, in the bulk harvested material, in 5 out of 6 cases had significant reductions in protein content. In the disease-ranked material, Span had consistent significant reductions in protein content, while Zephyr had not.

The protein and oil content of the seed were used to calculate the protein content of the meal. As expected, where significant reductions in oil and protein content of the seed occurred, a significant reduction in protein content of the meal was found. Span was most consistently reduced in protein content of the meal. Correlations on these quality characteristics indicated that protein content of the seed and meal had the strongest, positive, significant correlations. In the bulk-harvested material both protein of the seed and meal correlated significantly and positively.

Fowler and Downey (1970) indicated that the oil content of rapeseed accumulated fairly rapidly from 14 to 28 days after pollination (DAP), but from 35 DAP to maturity, oil deposition occurred at the same rate as dry matter. Since, Span and Zephyr infected with black spot matured from 7 to 12 days before the non-inoculated controls, the reduction in oil and protein content may be caused by the shortened period of deposition time for oil and protein.

Wetter (1965) showed that the major components of seed in Argentine and Polish varieties were oil, protein, carbohydrates, and crude fibre (lignin and insoluble carbohydrates). Thus, if the oil and protein content of the seed were reduced, there probably was an increase in the carbohydrates and crude fibre of the seed. This, in itself, detracts from the value of the rapeseed meal (Young, 1967).

The major fatty acids in the oil were not greatly affected by black spot of rapeseed. The only significant changes that occurred were in the palmitic and linoleic acids of the oil. In Zephyr, bulk-harvested material inoculated with A. raphani, linoleic acid was decreased. In Span, disease-ranked material inoculated with A. brassicae, both palmitic and linoleic acid content of the oil was increased. The average figures for the bulk-harvested Zephyr for the control and three different fungal treatments indicated that linoleic acid content was decreased by A. raphani, increased by A. brassicae, and held relatively constant by the combination of both species. This suggested that the different fungi could affect the same metabolic process different ways.

The increase in palmitic and linoleic acid content in Span could have a relatively simple explanation. The correlations for Span, demonstrated that palmitic acid content and yield were negatively correlated. Fowler and Downey (1970) showed the greater the period of oil production, the lower the palmitic acid content. Since black spot caused early senescence, palmitic acid should be higher in the diseased material. The data for bulk-harvested Zephyr, however, suggested that a much more complex physiological interaction could be occurring. Oleic acid was found to correlate negatively with palmitic, linoleic, and linolenic acid the last three of which all correlated positively with each other. The observed correlations for the fatty acids with each other had been reported before (Stefansson and Storgaard, 1969). One interesting factor was, that in the disease-ranked material, the correlations were stronger with more significant values in the severe group than the control group in Span.

The glucosinolates present in the meal were not significantly affected by black spot, except for the disease-ranked material of Span inoculated with _A_. _brassicae_. As this group had the greatest yield reduction, an effect on the glucosinolates would be expected. The major isothiocyanates BI and PI were significantly reduced by the disease. In diseased-ranked material of Zephyr inoculated with _A_. _brassicae_, there was a definite trend for reduction of the OZT content in the meal. Kondra and Downey (1969) had done a study on the glucosinolate content of developing rapeseed. They found that the shorter the period from pollination to maturity, the lower would be the glucosinolate content. Thus, in diseased rapeseed plants, early maturity could have caused the lower glucosinolate contents.

The correlations within the glucosinolates indicated that in Span, BI and PI (the major glucosinolate hydrolysis products) were significantly and positively correlated; as were the minor glucosinolate hydrolysis products MeSB and PhE. But, there was a significant negative correlation between the two groups. This could be expected, as the minor glucosinolates have been considered precursers of the major ones (Kondra, 1973; private communication). The glucosinolates did not correlate consistently with yield or protein content.

A comparison of the yield reductions caused by A. raphani, A. brassicae, and a combination of both species in the bulk-harvested material and in the severe group of the disease-ranked shows that the reductions in yield were much greater in the severe group of the disease-ranked than in the bulk-harvested material. The reason for this would be the bulked material contained moderately as well as severely diseased material. In the disease-ranked material for yield, 1000 Kwt., and seed volume, the severely diseased plants were consistently and significantly reduced in 17 out of 18 experiments, moderately diseased plants in 6 out of 18, and slightly diseased plants in 2 out of 18. Protein content of the seed and meal and oil content of the seed in the disease-ranked material were significantly reduced in the severely diseased plants 8 out of 18 times, moderately diseased plants in 7 out of 18 times, and slightly diseased in 4 out of 18 times. The progressive effect of black spot disease in yield, 1000 Kwt., and seed volume was quite evident.

For the quality parameters, although there was a trend for progressive losses they were not as great as for the yield parameters. The disease-ranking had value, but it would have been better with fewer categories. The use of these categories such as healthy or control, slight to moderate, and severe would probably have given more definitive results.

In the bulk-material, the correlations indicated there were significant, positive relationships between yield, 1000 Kwt., seed volume, and protein content of the seed and meal. But, the comparisons of the correlations of the control group to the severe group in the disease-ranked material, were not that simple. Black spot disease of rapeseed appeared capable of changing the relationship of the parameters. Two examples were yield and seed density where a negative correlation was changed by the disease to a positive one, and palmitic and linoleic acid where the diseased changed the correlation from a significant positive correlation to a non-significant negative one. This indicated a complex physiological stress was placed on the plant by the black spot disease.

In the toxin study, the characteristic lesion of Alternaria species on leaves occurred on rapeseed affected by black spot caused by A. raphani and A. brassicae. Filtrates of A. raphani and A. brassicae were found to contain toxic metabolites which could cause similar foliar symptoms. The symptom, not as readily seen on leaf lesions caused by the fungal spores, but quite common with the filtrates, was the browning of the veins on the leaves. This browning of the veins has been a phenomenon that has occurred with other Alternaria species on their hosts (Templeton, 1972). Some of the toxic metabolites of A. raphani and A. brassicae were found to be heat labile, and on autoclaving their activity was destroyed, thus retarding the development time of subsequent symptoms. In general, Alternaria toxins have not been heat labile but Torikata (1972, private communication) has found evidence of a heat labile toxin from A. kikuchiana on pears.

The A. brassicae filtrate was more severe than the A. raphani in affecting Zephyr leaves. This followed the field situation, where A. brassicae caused more severe effects than A. raphani. In the toxin study, whether in concentration or in kind, the pathogenic fungi definitely produced toxic metabolites which were more damaging to rapeseed than did the saprophytic isolate, A. humicola. The ability of Alternaria species other than the pathogenic one to produce a toxic metabolite against a specific host has been shown before (Fulton *et al.*, 1965). Differences in pathogenecity have not only occurred between *A. raphani* and *A. brassicae* (Taber, 1964; and Changsri and Weber, 1963), but differences in pathogenecity have occurred within a species (Atkinson, 1950; and Van Schreven, 1953). The possibility that the production of toxic metabolites can contribute to greater pathogenecity of an *Alternaria* species on its host has been demonstrated before (Torikata, 1967). In this study *A. brassicae* appeared to produce more toxic metabolites and to be more pathogenic on rapeseed than did *A. raphani*.

In general, the results obtained from the various parameters, except yield, were not as consistently affected by the black spot disease in Zephyr as they were in Span. It appeared that yield, seed weight, and seed size were more strongly affected by the disease than the quality factors. The quality factors were usually affected adversely by black spot. However, the major exception was, that glucosinolates were reduced in quantity, thus improving the quality of the meal. Further, it appeared that the black spot disease placed a complex physiological stress on the plant, the effect of which depended on the *Alternaria* species present.

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